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The time-bomb ticks on

IN 1969 the Institute of Race Relations in London produced a massive report on race in British society, and in particular the problem of the coloured immigrant. *Colour and Citizenship* was a mine of information, not all of it very palatable. It also had some strong recommendations to make on the subjects of education and employment.

Broadly, the report recommended that the Race Relations Board "should make full use of its powers to initiate investigations". It continued, amongst other things, to say, "it is essential for a non-discriminatory employment policy that records be kept which distinguish employees by ethnic origin." This would be seen by some as discriminatory in itself, but there was felt to be no other way to determine whether minorities were afforded equal opportunities.

Five years later, the House of Commons Select Committee on Race Relations (in a report issued last week) is still loath to grant the Race Relations Board legal powers to initiate investigations, being dissatisfied with the preventive work it has done so far.

But how does all this affect the scientist; surely this is a matter with which he may have some general liberal sympathy but is it not really an affair for the industrial shop-floor?

About 2% of the population of Britain is coloured immigrant or of immigrant descent. West Indians comprise half this figure, Indians and Pakistanis another 40% of it. By 1986 the 2% will be up to 3% or maybe 4%, depending on immigration policies and fertility. It is erroneous, even now, to assume that the distribution by age of this section of the population is such that the children of coloured immigrants are not yet at an age at which others seriously consider further education, say 16 to 21. The percentage of immigrants in this age bracket is close to the percentage of the general population in the same bracket. Two percent of those eligible, in terms of age, for a university education are immigrants or of immigrant descent.

The next question is, obviously, how many are getting a university education, and it is at this stage that problems arise. Every university campus is, of course, a very multiracial affair but many of those contributing to this are temporary immigrants, in Britain for educational purposes. It is impossible to get statistics about permanent immigrants at university; these figures do not exist, indeed some university registrars expressed surprise that we should wish to know the numbers, as in order to be scrupulously fair they could not possibly ask of candidates for admission anything more probing than their place of birth.

This seems a wrong if well intentioned policy, and the sooner it is rectified in universities the better; the select committee has come to a similar conclusion in the more general fields of employment (duplicating the 1969

proposals of the Institute of Race Relations).

Those who do not learn from history are forced to relive it, and there are the clearest lessons from recent American history. American experience—with a much larger minority group population—is that passivity in matters of race relations leads to greater alienation. It was simply not good enough to bemoan the lack of suitably qualified applicants and place the blame elsewhere—in the schools, in the social system or whatever. Accordingly the whole process of acquiring staff as well as students in many American universities, and particularly those with government contracts, is a complex business of satisfying law enforcement agencies that positive steps have been made to recruit minorities. This is a tedious process in many ways, and is both irritating to the academic community and somewhat humiliating to the minorities involved. On the other hand, blunt as it is, it is the only tool available to ensure that at least those who have some aspirations towards higher education do get reached.

It would probably be wrong at present to go for measures as drastic as this in Britain if there is any hope that lesser measures can succeed, and there may be a few years left before alienation is so total amongst some immigrants that the thought of a university education is anathema. But the next few years will require some positive action—one idea widely supported is that since many immigrants only discover in their twenties that they have unrealised potential for which higher education would have been beneficial, universities should come to terms with a fairly regular intake of more mature immigrant students. Another need expressed by workers in areas of high immigrant numbers is for more people from places of higher education to visit their community, not to announce that they are lowering standards for immigrants, but to give potential students the challenge of raising their own standards.

100 years ago



LADY BARKER'S "LESSONS ON COOKING"

First Lessons in the Principles of Cooking. By Lady Barker (London: Macmillan and Co., 1874).

IN this little volume the authoress has proved beyond all manner of doubt how completely she is the right woman in the right place. Surely nowhere could the Committee for the National Training School for Cooking have found a lady superintendent better fitted than Lady Barker to put life and spirit into the scheme which they advocate, or one more thoroughly qualified to train and marshal the feminine bands that are now being drilled under her supervision in the South Kensington Schools of Cookery to invade and revolutionise the kitchens of the future in every part of the empire.

From *Nature*, 10, 283, August 13, 1874.



The unendangered whale

Dr Ray Gambell, of the Whale Research Unit at the British Museum (Natural History), discusses the new rules for whaling adopted by the International Whaling Commission this year and argues against previous calls for a complete moratorium.

EVER since the 1972 Stockholm conference on the human environment there has been a persistent call to impose a 10-year ban on commercial whaling. This year's annual meeting of the International Whaling Commission (IWC), held in London in June, again considered this proposed moratorium. The IWC's Scientific Committee advised that because the concept of individual species management is now operative, there is no biological requirement for a blanket ban on all catching, and indeed, catching of some species may be necessary to promote the maximum rebuilding of other depleted stocks because of interspecific competition.

The IWC, which regulates about 90% of the world's whaling, therefore adopted an amended moratorium proposal which sets out the criteria by which the various whale stocks will be harvested in future. Briefly, these divide whale stocks into three categories; 'protection stocks', which will not be hunted at all; 'sustained management stocks', which can be caught at carefully controlled levels; and 'initial management stocks', which are very abundant and can also be taken in suitably regulated numbers to prevent any risk of over-exploitation.

By adopting this regime for future commercial whaling activity, the IWC has effectively placed the regulation of whaling in the hands of its Scientific Committee. This committee is charged with the responsibility of allocating the various whale stocks into the three categories defined above, and then deciding the level of catch which each can sustain if it is available for exploitation. The original Convention for the Regulation of Whaling signed in 1946 was formulated to provide for the conservation, development and optimum utilisation of the whaling resources. This was to be based on scientific findings, but could also take into account the interests of the consumers and the whaling industry. In the past the

economic arguments of the latter two groups often outweighed the advice given by the scientists, with the consequent decline of some of the major whale stocks. Now the emphasis has swung very much towards the scientific side. It remains to be seen just how well the scientists can resist the political and economic pressures which up till now have been exerted mainly in the full commission meeting rather than in the Scientific Committee.

The present scheme for the management of the whale resources can be seen as part of the general development of the policy adopted by the IWC in recent years. This is based on the concept of sustainable yield. In a stable, unexploited population the number of new recruits exactly balances the deaths due to natural mortality. When the population size is reduced by whaling, the recruitment rate increases through a lowering in the age at which the animals become sexually mature, and also by an increase in the pregnancy rate. The natural mortality rate also falls as whales are caught before they have a chance to die naturally.

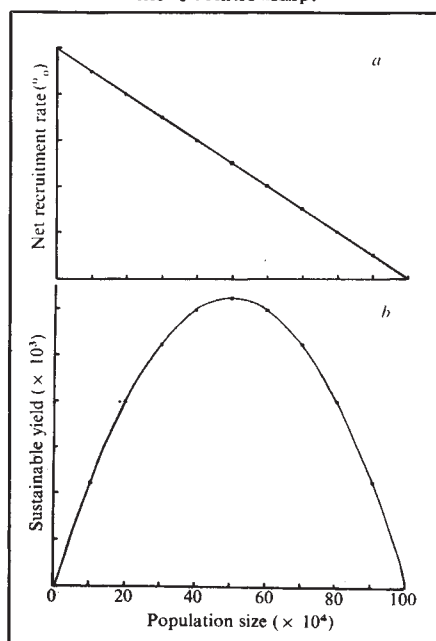
The exact forms of the relationships between the changes in recruitment rate and natural mortality rate with decreasing population size are not known for

certain in the whales, although there is some evidence from blue, fin and sei whales that the recruitment rate increases linearly. In Fig. 1a such a linear form for the variation in a theoretical net recruitment rate with population size is shown. This represents the gross recruitment by reproduction less the natural mortality. Multiplying every population size by the appropriate net recruitment rate gives a number, plotted in Fig. 1b, which is the surplus of recruitment over natural deaths. This surplus is a yield which can be harvested indefinitely without changing the overall population size. As can be seen from Fig. 1b, the yield is small in populations which have been much depleted in size. It is also small in those populations which have been but little reduced. The yield reaches a peak, the maximum sustainable yield (MSY), at some intermediate population size which is generally around half the original population number. Catching the sustainable yield will hold a population at its present level. Catching less than the sustainable yield will mean an addition to the total population size, which will therefore increase in numbers. For a population which is smaller than the level giving the MSY, this will allow some rebuilding towards that level; a population above that level will increase still further. Conversely, catching more than the sustainable yield will deplete the population, and for a population already below the MSY level will make it still less productive.

It is relevant now to consider where the various whale populations stand in relation to their MSY positions. In both major whaling areas, the Southern Hemisphere and the North Pacific, the same species are in very similar states. Blue, humpback and right whales are very seriously depleted and far to the left in the yield diagram (Fig. 1b). This is why they are totally protected from catching by the IWC. Grey whales in the North Pacific are also protected, although they have recovered from a very low level and now seem to be stabilised near their former original number.

Sei whales are close their MSY levels in both regions, although there are some variations between the component stocks in each case. Male sperm whales are also close to their MSY levels, but the females are relatively unexploited and far to the right of the yield diagram. Antarctic minke whales are also very

Fig. 1 *a*, A theoretical relationship between the net recruitment rate and a whale population size. *b*, Sustainable yield at each whale population size derived from the theoretical recruitment relationship.



The end of the line.

Copyright: National Institute of Oceanography.

abundant, as they have only been caught in large numbers in the past two seasons.

Fin whales, although a major component of the whaling industry's economy, are only at a third or a half of their MSY levels, and so need to rebuild in both regions.

Since 1965 the IWC has had the stated intent of setting the catch limits below the sustainable yields as calculated by continuing assessments. At that time the catch limits for the Antarctic baleen whale were set in terms of Blue Whale Units (BWU). One BWU equalled 1 blue whale, 2 fin whales, $2\frac{1}{2}$ humpbacks or 6 sei whales. These values were based on the relative oil yields of the different species, but the system was unfortunate because it took no account of the fact that each species may need a different degree of protection. The same criticism can also be levelled at the idea of a total moratorium on whaling, for not all whale stocks require such protection.

Catches of whales in the North Pacific have always been limited on a species basis, and from 1972 the Antarctic catch limits have also been set species by species. An even finer degree of control would be achieved by regulating the breeding stocks independently, but these cannot yet be fully identified. As a move in this direction, though, the IWC has now subdivided the Antarctic into three areas, and imposed maximum catches for each species in each of these areas.

Sperm whales, the largest of the toothed whales, did not come into the BWU system. In 1972 the Scientific Committee recommended that the catches of this species should be limited by each sex separately. This is practicable because the male sperm whales grow considerably larger than the females. The females had been protected from much catching by a minimum size limit. The Scientific Committee therefore also recommended that this size limit should be reduced, so that more females could be caught to bring the sexes into better balance. This is reasonable with the total catch limits imposed, which include a subdivision of the Southern Hemisphere into three areas with separate limits for the sexes in each.

The overall catch limits set this year are summarised in the Table, together with the estimates of sustainable yields. It is apparent from all this evidence that



the catches will not cause any species to be in danger of becoming extinct. Even the fin whale, which is the most reduced of the major species still hunted, should be rebuilding slowly. In addition, the IWC is committed to stop the capture of all fin whales in these two regions at least by 1976, so that the populations will then increase at the maximum rate possible.

The Scientific Committee will hold a special meeting to consider the assessment and status of all whale stocks in time to advise the commission next year on these matters. In addition, it will put forward detailed plans for extensive monitoring and research on the stocks. It is hoped that the costs of these projects, running into several millions of pounds, will be met by the United Nations (UN) Environment Programme, the UN Food and Agri-

cultural Organisation and other similar agencies.

There is much research needed. Up to the present, each whale species has been considered in isolation, without regard to the other whale species or the rest of the environment. Evidence of interactions is now becoming available. In addition, concepts involving the biomass of whales rather than their numbers will require study before they can be incorporated into the sustainable yield models already employed.

At all events, the future for the whales looks bright. There is no danger of any of them disappearing through overexploitation under the present controls, and there is the prospect of management being grounded on rational policies designed to provide the greatest long term benefit from this considerable natural resource of the oceans.

Overall catch limits set by the IWC and the estimated present sustainable yields of the whale populations

	Antarctic		North Pacific	
	Quota	SY	Quota	SY
Fin	1,000	3,200	300	750-900
Sei	4,000	5,200	2,000	2,500
Minke	7,000	7-12,000	—	—
Sperm* ♂	8,000	8,500	6,000	6,200
	5,000	4,500	4,000	1,700

*Whole Southern hemisphere.

international news

Two years ago a massive research programme on cardiovascular disease was launched in the United States when Congress passed a bill inelegantly entitled the National Heart, Blood Vessel, Lung and Blood Act of 1972. Modelled on the rhetorically overburdened campaign for the conquest of cancer, the research programme has received enthusiastic endorsement by President Nixon, who has repeatedly reaffirmed the administration's commitment to find ways of reducing the death toll from heart disease—"the nation's number one killer". But an expert committee which is supposed to keep an eye on this frenzied research programme has charged that the effort so far has been grossly underfunded and badly misdirected. In short, the Administration's commitment has been long on rhetoric but short on dollars.

The criticisms were made in a report to Congress by the National Heart and Lung Advisory Council which was actually written in December last year but which has spent the past seven months being reviewed by the Office of Management and Budget and the Department of Health, Education and Welfare. President Nixon finally passed the document on to Congress last week with the message that it "merits serious consideration", although it contains recommendations which are "at variance with the Administration's views".

Chief among those recommendations is that the National Heart and Lung Institute (NHLI) should be given a budget this year of \$520 million, instead of the \$309 million proposed by the Administration. The council charged that the amount of money being spent on cardiovascular research was actually

Heart research short on cash

by Colin Norman, Washington

less last year than in 1968 if inflation is taken into account, and it reported that "the resources for this accelerated attack [on heart disease] have not been forthcoming".

Such complaints have come as no surprise to the Administration, or to anybody else for that matter, since the council is composed chiefly of scientists directly or indirectly engaged in research on cardiovascular disease, and the report thus has all the appearances of special pleading by a section of the biomedical community. Although the Administration has so far made no formal public response to the council's recommendations, officials point out that the National Heart and Lung Institute (NHLI) has in fact fared relatively well. Since this year the Administration proposed a hefty increase in its budget (\$25 million) while all the other institutes at the National Institutes of Health with the exception of the National Cancer Institute either had their budgets cut or kept static.

Nevertheless, it is difficult to deny that much of the proclaimed attack on heart disease has gone off at half cock, at least when it is measured against the intentions of the 1972 Act. The Bill suggested that \$460 million should be spent by NHLI in the 1974 fiscal year—which ended on July 1 this year—and that \$520 million should be spent in the 1975 fiscal year. Only \$280 million was

spent last year, however, and the Administration has proposed a budget of \$309 million for 1975.

Aside from the question of funding, the council also takes the Administration to task for a variety of other sins, chief of which is the move early last year to abandon the policy of providing fellowships and traineeships for biomedical scientists. Although the council neglects to mention that in July last year the Secretary of Health, Education and Welfare, Caspar Weinberger, announced that the Administration would not scrap the training programmes after all, it states that the unavailability of funds for biomedical training "has reduced the research manpower and the productivity of many of the very best research groups supported by the National Heart and Lung Institute." (Last month, Congress finally approved a bill which will more than double the Administration's proposed expenditure on biomedical research training. Administration officials said last week that they are looking at ways to implement the bill, and presumably that will still the criticisms of the council.)

The council also comes up with a clutch of recommendations for endowing chairs at universities for professors engaged in cardiovascular research, for maintaining a good balance between grants and contracts, and for establishing special research and demonstration centres around the country. Another prominent suggestion is that Congress should establish minimum qualifications for members of the council itself; this is believed to be a reference to the fact that the singer Frank Sinatra was appointed to the council last year, but failed to attend any of its meetings.

A SOUTH AFRICAN gynaecologist is pioneering a technique for fallopian tube transplant operations which may offer a better answer than *in vitro* fertilisation for childless women.

For *in vitro* fertilisation to take place the egg must be caught at the precise moment of ovulation, and since ovulation can occur at any time, doctors must be on constant alert and time-consuming observations of the woman is needed. By contrast, the suggested transplant technique is much simpler and cheaper, because after a short operation nature can take its course.

According to Dr Abraham Rubin, he and his colleagues at the University

Transplant babies?

from Ian Ridpath

of the Witwatersrand Medical School in Johannesburg are hoping to start soon an experimental programme that will lead to the first operation of this kind in women.

Fallopian tubes for transplant are readily available from women who have had a hysterectomy. Substituting them for diseased tubes is a relatively simple operation that is little more than an extension of existing surgical techniques regularly used by gynaecologists. Successful fallopian tube

transplants have already been performed on sheep by Dr M. Katz at the University of Cape Town, and Dr Rubin thinks that the same technique can be extended to humans.

It would be particularly valuable where infection of the fallopian tube is common, says Dr Rubin. Unlike other forms of transplant there are no worries about rejection, since the technique is to use the tube for one cycle and then it can be removed. Rejection only becomes a problem after three to four weeks. Donors do not therefore have to be specially selected, which helps to make the operation more widely available, and Dr Rubin envisages it being performed regularly.

Geologists as professionals

from Peter J. Smith

PHYSICISTS, chemists, biologists and mathematicians in Britain have their respective professional institutes, and there are no less than 15 chartered institutions catering for the needs of the various types of engineer. British geologists, by contrast, have no professional body, although members of certain subdisciplines within the earth sciences are eligible to join appropriate existing organisations (some geophysicists, for example, are members of the Institute of Physics). So is there a case for the formation of a professional Institute of Geology? A working group sponsored by the Geological Society believes there is, and in its published report (*Report of the Working Party on Professional Recognition*, Geological Society of London, 1974) recommends that the degree of support for a professional organisation in geology be widely tested.

The Geological Society itself is a learned society, but has lately been finding it difficult to reconcile its traditional role with the need to represent British geology to the outside world. As an example of this need, the report cites the setting up in 1973 of the Council of Environmental Science and Engineering by the two councils representing, respectively, the professional science institutes and the chartered engineering institutions. Because there is no professional body of geologists affiliated to the two sponsoring councils, geologists have no formal participation in an organisation concerned with environmental problems.

Because earth science is one of the two branches of science most directly concerned with the environment, the need for a 'voice of British geology' is clearly much more than a parochial academic matter. The Geological Society working group has therefore examined in some detail the case for a professional body which "would be expected to provide representation of geological interests at different levels". But external representation is not the only, nor perhaps even the principal function of a professional institute; and it is envisaged that the proposed Institute of Geology would also operate a code of ethics and professional conduct, set professional standards for the various categories of membership, provide advice on career structures and personal security, and disseminate information on general and professional matters.

But if the idea of an Institute of Geology (by this or any other name) comes to be widely accepted, how could it be put into practice? The re-

port considers five "alternative [sic] pathways" (in addition to maintenance of the status quo) but in the end rejects four of them as unlikely to succeed. The first—the creation of a professional class of Fellowship within the Geological Society—was rejected at an early stage in the discussions because although it would strengthen the society financially and allow it to speak for professional geology nationally, it would probably lead to a dissension among the ranks of the non-professional geologists (the "second class Fellowship"). Moreover, there is some doubt as to just what professional services the society would be allowed to offer under the terms of its Charter; the lack of significant additional services recently led to the failure of a similar scheme in South Africa.

Indeed, the Charter seems to be a major stumbling block, for both it and the society's grace-and-favour apartments might also be put at risk by two other possible schemes — a more thoroughgoing conversion of the society into a professional-cum-learned society (a solution adopted by the physicists) or the creation of a new body under the society's direct sponsorship. This then leaves just two other possibilities—the creation of a new body by independent sponsorship, either with no society participation or with the society's general support. The first of these is rejected because of the high costs arising from the need for administration and accommodation separate from the society and because of the possibility of conflict with the society. In the end, therefore, the working group recommends independent sponsorship with society support.

The working group is now soliciting views from as many geologists as possible, both on the idea of a professional body as such and on the proposed structure as set out in an appendix to the report. Those who respond should pay particular attention to the latter, for experience suggests that detailed organisational points which appear unimportant can, in fact, have profound consequences. For example, the working group suggests, without discussion, that the new institute should "be established as a charitable body" — an apparently innocuous enough statement. But under the present, admittedly chaotic, laws governing charitable bodies in Britain this would prevent the institute from taking part in any activity remotely political. Registration as a charity would therefore place constraints on the institute which are in many ways comparable to those now acting on the Geological Society itself. Would this be in the best interests of the geology profession?

Finally, there is at least one surprising omission from the report. The

working group makes the contentious point that "the science of geology embraces many diverse fields including . . . geophysics . . .", but fails to consider, or even mention, the anomalous position of British geophysicists within the Royal Astronomical Society. Although the working group does not regard itself "in any sense as representative", it would surely have been sensible to have had a geophysical representative of that society on the group right from the start.

Ispra's 'factor up' energy programme

THE energy crisis has done a service to both the solar and hydrogen energy projects at the largest of the European Community's Joint Research Centres, that at Ispra on Lake Maggiore, originally an exclusively Euratom establishment. The aim of the solar work, which was only initiated in 1973, is to probe promising areas not well covered either by industry or national research establishments in member countries, and a substantial increase in funding is expected this year.

The approach may be described as a 'factor-up' programme. Fundamental to work at Ispra is that solar energy—although abundant and nonpolluting—is both diffuse and intermittent. Accepting that it is already possible to heat a house by solar energy—it is being done in the French Pyrenees through air convection generated in hollow blackened walls—Ispra is investigating higher efficiency systems. For domestic heating it is pursuing a plug-in, fixed-angle collector unit (not a house) capable of generating 100° C where present collectors do not surpass 65° C, largely because of heat loss from the surface of the collector. Small scale tests of one method are in progress and these involve adaptation of the 'anti-radiation' cells developed by Francia.

A temperature of 100° C is a threshold value for another small scale 'domestic' application of special interest in underdeveloped arid regions. "To use the Sun to pump water out of the desert" as the brisk energy director at Ispra, Dr Joachim Gretz puts it. A heat source of this order could be coupled to a 1-kW power set able to pump up water for 8 hours a day (during sunlight) for human and animal subsistence. Dr Gretz points out that in many areas primitive concepts of the natural world mean that communities are dying of thirst while there is pumpable water only 16–60 m down.

Looking further ahead Ispra sees real promise in quantum devices with which yields of 1 kW m⁻² would be possible through photoelectric or photochemical conversion. □

correspondence

Victimisation

SIR,—In your issue of July 12 you publish the appeal of Dr Peleska, the Czechoslovak scientist, to the World Federation of Scientific Workers.

Unfortunately, the WFSW has many allegations of victimisation against scientific workers brought to its attention, involving many different countries. For example, in the same letter as that in which I reported the receipt of Dr Peleska's letter to the vice-presidents of the federation, I also had to report two other allegations, in some respects even more serious. There was the case of Professor José Ferreira de Alencar, a Brazilian anthropologist and sociologist, who was said to have been imprisoned and ill-treated as a direct consequence of reports he has written on the social status of the people of North-East Brazil. There was also the case of Professor Horst Holzer, one of the leading sociologists in the Federal German Republic, who was dismissed in April 1974 by the Bavarian Ministry for Education and Culture because of his membership of the German Communist Party.

There is no doubt of the policy of the World Federation of Scientific Workers on the question of the right of scientists to work. It is, for example, dealt with in the Declaration on the Rights of Scientists, adopted at the Ninth General Assembly of the Federation in Paris in 1969. Thus:

"3.2. Scientific workers should have the right to work in accordance with their scientific capacities and Governments should endeavour to ensure this right.

"3.5. Scientific workers should have equal rights in their professions, regardless of sex, race, nationality, creed or political conviction."

We have affiliated organisations of scientific workers in 30 countries and they all accept these basic principles of the Federation. When a serious *prima facie* case of victimisation of a scientist is brought to our attention we refer it to our affiliated organisation if there is one in the country concerned, ask them to look into the matter and, where appropriate, either take action themselves or suggest possible action the Federation can take. In some cases in which we have intervened in this way appropriate remedial action has been taken. In other cases new circumstances have been revealed

which have put a different complexion on the case.

It is difficult to see what further action is open to us. The preamble to our Constitution states quite clearly: "The Federation will endeavour . . . to develop relations between scientists having regard to the autonomy of each organisation, to the equality of rights, to the avoidance of interference in the affairs of national organisations." It is difficult to see how an international body could be based on any other principle. Differences of practice between different countries are very great and appropriate allowance must be made for this. Very many useful initiatives have been taken by the Federation and the close contacts maintained between our affiliated organisations in different countries are beneficial to scientists and for science itself. Our aim must be to strengthen these contacts.

Yours faithfully,

E. H. S. BURHOP

World Federation of Scientific
Workers,
London, UK

Ill reactor?

SIR,—The United Kingdom is now not only a *de facto* but also a *de jure* partner in the Institut Laue Langevin, built around the high flux neutron beam reactor. Since we are equal partners in the running and use of the establishment, we might also claim an equal share in its name. Fortunately, the choice of a suitably alliterative British scientist is obvious. What could be better than to call it the Institut Laue Langevin Lonsdale. Such a choice may be justified on several grounds:

Kathleen Lonsdale was a most distinguished scientist, the first woman elected Fellow of the Royal Society. She was a crystallographer, hence her name has obvious associations with the high flux beam reactor.

Just as von Laue and Langevin, Kathleen Lonsdale was all her life a champion of dignity and freedom.

Changing ILL to ILLL would have the advantage that newspaper headlines could no longer be misunderstood, and there would be no danger of an announcement like "Ill Deputy Director Returning to England" causing anguish to Dr W. M. Lomer's friends.

Yours faithfully,

N. KURTI

Clarendon Laboratory,
Oxford

Anonymous refereeing

SIR,—It seems that the best way to obviate the misuse of the unilateral anonymity granted to reviewers is to extend anonymity to authors as well. When the reviewers get a paper from the editor but have no idea who the authors are or what their affiliation is, they would find less pleasure in making unnecessary and uncivilised remarks. In addition, the reviewers would be able to judge a paper more justly and without prejudice.

This bilateral anonymity programme is simple, and can work. And yet, after this is adopted I should like to go one step further in proposing that all papers be not only reviewed but also published anonymously.

The idea "publish or perish" did stimulate scientific research for a while. But it has now come to the stage where too much energy is wasted in unnecessary publications. I believe most scientists would agree that the total amount of papers published yearly could very well be cut 50% or more without showing any significant impediment in the progress of science. If this immense waste is allowed to continue, the advancement of science will actually be slowing down rather than moving faster. The main reason for this is that we place too much emphasis on the number of papers that a scientist has produced. One obvious result has been much unnecessary repetition.

If all papers were published anonymously, then the "status" of being a prolific writer would be diminished. The fight among authors about whose name should appear first would disappear. The scientific community could, furthermore, judge a paper solely on its merit.

My ultimate hope is to dissociate totally the name from the achievement. If Einstein were alive today, I believe he would not mind if people discussed and utilised the great theory of relativity without mentioning or even knowing his name. But I dare not advocate such a radical proposal at this time. Scientists are human, and as such must be selfish beings after all.

Yours faithfully,

TA-MING-FANG

Division of Engineering and Applied
Physics,
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news and views

Older comets lose their sparkle

ON each successive approach to the Sun a comet becomes fainter. This phenomenon can be explained as follows. The nucleus of a comet can be considered as a conglomerate of ices and dust, and the regular heating of the nucleus each time the comet moves through the perihelion position of its orbit leads to sublimation of the ices, the copious emission of volatiles, a concomitant loss in mass and either a reduction in the size of the nucleus or the production of a relatively ice-free, porous dusty mantle around the nucleus. Thus, the emission of volatiles decreases each time that the comet goes past the Sun and the maximum brightness of the comet therefore diminishes. Since the 1920s, however, there has been a considerable controversy as to the rate of this secular decrease in brightness. The protagonists today are Sekanina (Smithsonian Astrophysical Observatory, Cambridge, Massachusetts), Vsekhsvyatskij (Astronomical Observatory, Kiev University), and Kresák (Astronomical Institute of the Slovak Academy of Sciences, Bratislava). A recent article by Kresák (*Bull. astr. Insts Csl.*, **25**, 87; 1974) adds fuel to the debate, the main point of which is not the fact that short period comets (those with periods less than, for instance, 20 yr and with a median period of 6.8 yr) are short lived objects in Solar System terms, but whether their decay process has actually been observed as a secular decrease in brightness within the age of accurate astronomical observation, that is, the last century and a half. Sekanina and Vsekhsvyatskij think that decay has been observed; Kresák disagrees.

A principal difficulty is that photometric observations of comets, collected by Vsekhsvyatskij, have been used by other researchers without due consideration of the great hazards involved in comparing observations from different epochs between which instruments and observing techniques have both developed enormously. Use has sometimes been made of visual magnitude data quoted to ± 0.1 mag without any consideration of systematic errors which may amount to many magnitudes (magnitude measures brightness on a logarithmic scale; one magnitude is equivalent to a 2.5 fold change in brightness).

The head of a comet consists of a diffuse coma which surrounds a central condensation or nucleus. Observations made with comet seekers of low focal ratio and moderate magnification, or with small cameras, allow the intrinsic magnitude of the diffuse comet head to be compared with the point images of reference stars, without the introduction of appreciable systematic error. If, however, the focal length and exposure time are increased, the apparent motion of the comet is accentuated and the image is trailed. Furthermore, in a telescope with a long focus a typical comet head of several minutes of arc in diameter may cover the whole visible field of view, adding to the sky background, and leaving only the nucleus to be measured. The luminosity of the nuclear condensation may be as low as 0.1–1% of the total luminosity of the comet. During the past two centuries magnitude estimates using small instruments or the naked eye have been replaced by the application of large telescopes and astro-

photography. That improves the detection limit of point sources but only partially affects the detection of large and diffuse comets.

Kresák illustrates this problem by considering published observations of the 1962 appearance of Comet Tuttle-Giacobini-Kresák, which had a very small nucleus and a large coma. The observations show systematic differences: there is an intensity ratio of 1000:1 between the most divergent observations.

Incompleteness of observations also introduces a difficult problem: for example, the absolute magnitude (the magnitude that the comet would seem to have if it was placed in a standard position 1 AU from the Earth and the Sun) is not the only decisive factor effecting the probability of detection; there are also important geometrical conditions of a seasonal nature. If there is a Gaussian random walk in the absolute magnitude at each return, then the comet will most probably be discovered when the random fluctuation makes it brightest (thus, the first observation is non-typical). Moreover, as observing conditions determine whether or not it will be observed on subsequent returns, the actual observations will be biased. As the limits of detection have decreased with time because of instrumental improvements, minimum values which are detected easily now would, in previous decades, have been missed. Both these effects bias the data towards a rate of absolute brightness decrease which is too steep.

Kresák, using observations of the total brightness of the whole comet head, concludes that the secular decrease in brightness of the periodic Comet Encke is only 1 mag per century and not 3 mag per century, as was found by Sekanina (*Astr. astrofiz.*, USSR, **4**, 54; 1969). And the rate of decrease is not accelerating. Kresák checked his absolute magnitude results by using the maximum visual magnitude at each apparition and obtained a similar decrease.

A rapid secular decrease in the brightness of short period comets would imply that the objects observed today were considerably brighter in preceding centuries, assuming, that is, that they have not in the meantime been captured from orbits of large perihelion distance. Unfortunately, short period comets are mostly too faint to be recorded as naked eye observations in mediaeval and ancient records, and Jovian perturbations drastically affect their orbits, which makes backward extrapolation from today's observations exceedingly difficult. One exception is Comet Encke, which has a relatively stable orbit well inside that of Jupiter.

Ho Peng Yoke (*Vistas Astr.*, **5**, 127; 1962) produced a catalogue of ancient and mediaeval Chinese observations. A backward extrapolation of the secular brightness of Comet Encke would imply an easy identification of Encke in Ho Peng Yoke's catalogue; for example, extrapolation using Sekanina's value would make the comet frequently brighter than Venus before 1700. Kresák, however, found no evidence in the catalogue of periodic appearances of Comet Encke, and thus concluded that Sekanina's result is too large.

The high decay rates found by Sekanina and Vsekhsvyatskij have been applied by many authors to predict death dates for individual comets. Kresák shows not only that these projections would remove most of the well observed short period comets from our view within the

next decade or so, but also that many comets are still visible long after their predicted demise, two more facts in favour of a slow decay rate. Vsekhsvyatskij's decay figures of 0.3–0.4 mag per revolution gives short period comets a lifetime of two centuries, and proposed accelerations of this rate reduces this life to a few decades. Kresák finds that during the period 1930–1970 the discovery of short period comets has stabilised at a constant level of seven per decade. This perturbational capture rate is, in fact, no adequate substitute for the rapid loss predicted by Sekanina. If the short period comet population is in equilibrium, Kresák's low values of decay rate must be applicable.

Kresák concludes that the process of ageing of comets, and its effect on the variation in absolute brightness, cannot be satisfactorily explained by a simplified model of continuous mass loss which induces a continuous secular variation in absolute magnitude. Rather, observational evidence suggests strongly that the evolution proceeds stepwise, with short periods of abrupt evolution interspersed with long, relatively latent periods during which the brightness undergoes some fluctuation but in general remains essentially unchanged.

DAVID W. HUGHES

Dirac completes his theory of large numbers

It is not often that a man begins a new physical theory in his thirties, and then returns to finish it off forty years later. In a letter to *Nature* in 1937 (139, 323) Dirac added to his store of brilliant ideas a fundamental hypothesis concerning the values of various naturally occurring dimensionless numbers. In the past two years he has returned to this hypothesis to explore its consequences in detail. His latest article (*Proc. Roy. Soc. A*338, 446; 1974), deals with certain astronomical and cosmological topics.

Dirac's theory begins with the question: what is the age of the Universe expressed in some naturally occurring units? A fundamental unit of time is the interval required for light to cross a characteristic atomic dimension, say 10^{-13} cm. Then the age of the Universe comes out at about 10^{39} in these units. The significance of this number is that it turns out to be simply related to other large dimensionless numbers formed from astronomical and atomic data; for example, the total number of particles in the observable Universe is around 10^{78} , that is $(10^{39})^2$, and the ratio of the electric to gravitational attraction between an electron and proton, $e^2/Gm \cdot m_p$, is also about 10^{39} . In view of the fact that the first number obviously increases with time, the coincidences involved by these simple relationships seems great. Dirac's explanation of this curiosity is that all such large quantities are connected, so that as the first increases with time so do the others. This is already a radical departure from conventional physics, which in addition imposes severe restrictions on cosmology. Dirac calls this connection the large numbers hypothesis, in which he expresses "great confidence".

In his 1937 article, Dirac was led by this hypothesis to a model universe which expands with time t like $t^{1/3}$, making it about half as old as the more conventional big-bang models. In his recent work, however, the bald hypothesis is accompanied by a detailed gravitational theory which bears some similarity to the work of Hermann Weyl and E. A. Milne. A central feature of this work is the proposal

that there are really two space-time metrics; one, which is unmeasurable directly, enters into the Einstein equations (which remain valid) and the other is what is actually measured in laboratory experiments involving atomic apparatus. A connection between these two metrics is provided by a consideration of the motion of the Earth around the Sun, which in Newtonian approximation is essentially determined through the relation $GM = v^2 r$ connecting the Newtonian constant of gravitation G with the solar mass M , velocity of the Earth v and radius of the Earth's orbit r .

There then follows an argument which is typical of that used throughout Dirac's work. In terms of Einstein units, the quantities in this equation are constant. But in atomic units one must satisfy the large numbers hypothesis, so that G must decrease as t^{-1} in order that $e^2/Gm \cdot m_p$ increases proportionally to t . Theories involving a changing constant of gravitation are not new: for example, Brans-Dicke theory predicts a similar effect. The change is, however, very small and barely detectable with current technology.

Once again, the number of particles in the Universe $(10^{79})^2$ at present, is required by the large numbers hypothesis to increase as t^2 , which is interpreted by Dirac as a form of continual creation along the lines of the steady state theory.

Two possible creation mechanisms are proposed, with the new matter either appearing concentrated in existing masses (multiplicative creation) or spread out in the intergalactic spaces (additive creation). In the latter case the mass M in the above equation is constant, so that r in atomic units varies like t^{-1} . In the former case $M \propto t^2$ and $r \propto t$. It follows that the ratio of the two metrics can be t^{-1} or t , and that the Solar System must be either expanding or contracting with time.

In terms of Einstein units, if multiplicative creation is adding continuously to the mass M of the Sun, conservation of energy requires that the nucleons in the Sun decrease in mass as t^{-2} to compensate. Through the relation $e^2/Gm^2 \propto t$ this further requires $e \propto t^{-3/2}$ and $\hbar$ (Planck's constant) $\propto t^{-3}$. A number of interesting cosmological consequences follow. First Dirac argues that any universe consistent with the large numbers hypotheses must be static, to avoid the introduction of a characteristic cosmological epoch (that is a constant large number). The red shift of distant galaxies, normally interpreted as a recessional effect, is instead accounted for by the decrease in the unit of atomic time interval. The Universe is prevented from gravitational collapse by the introduction in Einstein's equations of the cosmological constant which may be non-zero for multiplicative creation because of the correct time dependence of the metric ratio in this case. Dirac thus arrives at a version of Einstein's original static model universe.

In the case of additive creation Dirac proposes to conserve energy by the introduction of an unobservable negative energy field spread throughout the Universe, along the lines of the C-field of Hoyle and Narlikar. The total energy density of the Universe thus vanishes, so that the global geometry is just the Minkowski space of special relativity. In comparing the two models, Dirac inclines to the latter, on the basis that the creation of new atoms in existing material would lead to insuperable difficulties concerning the crystalline structure of very old rocks.

Dirac's article is written in his usual lucid and direct style. The ideas it contains, a mixture of old and new, are probably unpalatable for most modern cosmologists. Yet they are the product of a lively imagination, challenging the fundamental principles on which modern theories of astronomy, cosmology and physics itself are founded. Coming from a physicist of Dirac's stature, that is at the very least thought provoking.

P. C. W. DAVIES

Passive transport of insect urine

from our *Insect Physiology Correspondent*

It has long been realised that the Malpighian tubules of insect constitute a filtration-reabsorption system comparable with that of vertebrates. But in the absence of a glomerulus the flow of water is sustained by secretion in the upper region of the tubule, as in the aglomerular kidneys of some fishes, and not by pressure of the blood. Reabsorption of water can occur in the lower segment of the tubules, and in the hindgut and rectum—as in the kidney tubules and cloaca of many vertebrates. As was first shown by Ramsay, the osmotic driving force for the flow of water is produced by the active secretion of ions, particularly potassium; although sodium and chloride can play a part in some insects. The wall of the tubules was believed to be readily permeable to small organic molecules, so that sugars, amino acids and the like entered along with the water; the valuable components were reabsorbed, while toxic materials and waste products were discarded.

This problem of the passive permeability of the Malpighian tubules to organic solutes has now been re-investigated by Maddrell and Gardiner (*J. exp. Biol.*, **60**, 641; 1974) in the blowfly *Calliphora*, the locust *Schistocerca*, the hawk-moth *Manduca* and the blood-sucking bugs *Triatoma* and *Rhodnius*. Some eighteen compounds, labelled with carbon-14 or tritium were used as test substances; mostly amino acids and sugars, but including urea, urea acid

and inulin. Most of the experiments were done on Malpighian tubules isolated *in vitro* in appropriate saline solutions; but the results were confirmed by *in vivo* observations. The concentrations of the test substance in the bathing medium (*M*) and in the secretory product (*S*) were measured, along with the rate of fluid secretion, which could be varied experimentally by appropriate stimulation. It was found that there was always a linear relation between the *M/S* ratio and the rate of fluid secretion—as was to be expected if the transport was purely passive, the slope of the line being determined by the permeability of the tubule to the substance in question. There was thus, for example, a very gentle slope for the small molecules of xylose, as compared with the steep slope for the large molecules of inulin. Highly charged molecules penetrate more slowly than those more nearly neutral electrically: thus L-valine accumulates at one fifth the rate of D-xylose. The permeability of the tubules to such materials as inulin (molecular weight 5,200) is surprising. In some experiments, where the tubules are secreting very slowly, inulin may reach a concentration in the secreted fluid nearly 50% of that in the bathing medium. The authors suggest that much of this passive permeability lies in the intercellular spaces (see figure).

These experiments seem to have established the fact that the content of small organic molecules in the Malpighian fluid is determined by a passive non-discriminatory process. This means that the onus for conservation and discrimination resides in the re-

absorptive system. Here one is less well informed. The reabsorption of ions by the rectal epithelium has been well studied; and some absorption of amino acids and sugars occurs here. But a large part of the reabsorption probably takes place in the Malpighian tubules themselves, notably in the lower segments, and perhaps in the ileum where this exists. Furthermore, not all 'secretion' by the tubules is passive: some materials such as biliverdin, are transported by a process of endocytosis and exocytosis; the excretion of indigo carmine, which was so widely studied in the last century, presents some curious features that are not fully explained.

Modified nucleotides in messenger RNA?

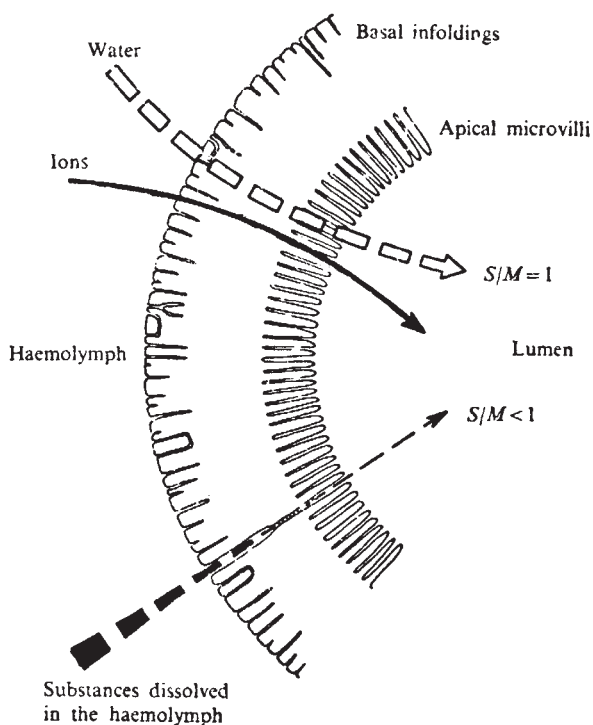
from Alan E. Smith

UNTIL recently it was thought that minor bases were present only in transfer RNA (tRNA) and ribosomal RNA (rRNA), but the difficulties in obtaining sufficient pure messenger RNA (mRNA) prevented definitive analysis of the latter. The presence of poly (A) in mRNA and heterogeneous nuclear RNA (hnRNA) can now be utilised to obtain purified fractions of these species and they can be examined for nucleotide composition without risk of contamination by rRNA and tRNA. In the first issue of *Cell* (**1**, 37–42; 1974), Perry and Kelly reported the results of such an analysis of mouse L-cell mRNA and showed that on average there are about 2.2 methyl groups per 1,000 nucleotides. This compares with about 13 methyl groups per 1,000 in rRNA, and means that an average cellular messenger molecule containing, say, 3,000 nucleotides has about 6 or 7 methyl groups.

hnRNA, on the other hand, contains many fewer methyl groups and Perry and Kelly therefore suggested that in eukaryotic cells methylation like the addition of poly (A) constitutes a post-transcriptional modification of mRNA precursors. These authors, however, did not identify the minor bases present in a specific mRNA species nor did they position them within the molecule.

The first identification and precise location of a minor nucleotide in mRNA has now been reported using a somewhat exotic system. In an elegant paper (*Nucleic Acid Reports*, **1**, 809–822; 1974), Furuichi shows that the mRNA of silkworm cytoplasmic polyhedrosis virus (SCPV) contains a 2'-O-methyl adenylic acid in the 5' terminal position, and that transcription to give mRNA is intimately coupled to the methylation reaction.

SCPV, like reovirus, contains a frag-



Schematic section of the wall of a Malpighian tubule to show the routes of transport of ions and water and diffusion of organic substances through the cell wall. *S/M* is the ratio of concentrations in the secreted fluid and in the bathing medium or haemolymph.

mented double-stranded RNA genome which consists of ten different segments. The terminal nucleotides of the double stranded RNA have recently been reported (*J. molec. Biol.*, **85**, 31–48; 1974) and the 5' nucleotide sequence of one strand of each of the segments is A*GU, where A* is a methylated adenosine. SCPV contains a virion-associated transcriptase which transcribes all ten double stranded RNA fragments *in vitro* to give mRNA molecules each of which begins with a 5' terminal sequence ppAG (*J. molec. Biol.*, **85**, 21–30; 1974; see also *Nature*, **250**, 13–14; 1974).

Since the activity of the SCPV transcriptase is low compared with that of the enzyme from reovirus, and because the genomic RNA is methylated, Furuichi tried adding a donor of methyl groups to the *in vitro* reaction mixture, and observed a huge stimulation of mRNA synthesis on addition of S-adenosyl-methionine (SAM). The product formed *in vitro* was found to be virus-specified and consisted of complete copies of one strand of each of the genome segments containing, on average, one methyl group per RNA molecule. The methyl group incorporated into the viral mRNA was detected in 7-O-methyl adenylic acid which is also present at the 5' terminus of the genomic double stranded RNA.

Furuichi examined the RNA made during very short incubations and found that methylation occurs early in the transcription process when the nascent chains are less than ten residues long. Since mRNA synthesis is almost totally dependent on addition of the methyl donor, and methylation is such an early event, Furuichi suggests that methylation may be involved in the initiation of mRNA synthesis and that SCPV transcriptase utilises a methylation-coupled transcription system, which is so far unique.

It will be of interest to establish whether other viral transcription systems are similarly coupled. It is already known, for example, that the RNA tumour viruses contain virion-associated methylase enzymes, as well as reverse transcriptase. So far the effect of SAM *in vitro* has not been reported in this system.

Of even greater interest will be an understanding of the initiation of hnRNA synthesis in normal cells. Bajzar, Samarina and Georgiev (*Molecular Biology Reports* **1**, 305–310; 1974) have isolated the 5' terminal nucleotides of mouse cell hnRNA and identified them as pppAp and pppGp. So far there is no evidence for methylation in these positions. It is possible, however, that methylation occurs at internal positions in hnRNA and that such modifications act as markers for subsequent cleavage reactions. It may be significant that

the chromatographic mobility of some of the methylated nucleotides detected by Perry and Kelly in mouse mRNA suggested that more than one phosphate group is present. Perhaps some of the methyl groups are attached to nucleotides (pX*p) which originate from the 5' end of mature cellular mRNA.

Models of magnetic anomalies

from Peter J. Smith

A MAGNETOMETER towed at or near the ocean surface will record the well known linear magnetic anomalies which arise from alternating normal and reversed magnetic material within the oceanic crust. A magnetometer towed along very close to (say, within 100–200 m) of the sea floor, on the other hand, records anomalies which are much narrower and yet much greater in amplitude. But to what is this fine structure due? Do these small scale variations within magnetic polarity epochs and events reflect real physical variations of internal crustal properties or are they merely the result of topography or some other equally uninteresting phenomenon?

Conclusions on this point seem to differ. Atwater and Mudie (*J. geophys. Res.*, **78**, 8665; 1973) measured near-bottom anomalies on the flank of the Gorda Rise and found that almost all of them could be accounted for by a uniformly magnetised basement with topography. But some years ago, Luyendyk (*J. geophys. Res.*, **74**, 4869; 1969) found such an explanation unsatisfactory in connection with the fine structure in an area on anomaly 10 west of southern California. Instead, he invoked variations in magnetisation which arise either from ancient magnetic field variations or changes in petrology with distance from the ridge crest. Similarly, Larson and Spiess (*Science*, **163**, 68; 1969) concluded from a profile across the East Pacific Rise crest that small scale anomalies within the Brunhes epoch are unrelated to basement topography, and again appealed to fluctuation in palaeointensity.

Insofar as these conclusions refer to difficult areas they are not necessarily inconsistent, for it is quite conceivable that apparently similar phenomena in different regions could be explained in different ways. On the other hand, it is clearly important to know whether a common observation may be dismissed as an effect of little significance or whether it is necessary to look for deep-seated physical causes. So to investigate this matter further, Larson *et al.* (*J. geophys. Res.*, **79**, 2686; 1974) have constructed and analysed magnetic

block models based on the volcanic basement profile actually obtained across the East Pacific Rise by Larson and Spiess—a profile carefully obtained by continuously and simultaneously measuring the depth of the magnetometer with an up-looking sonar, the height of the instrument above the sea floor with a down-looking sonar, and the thickness of sediment with another down-looking sonar.

The first model was given a constant magnetisation of $0.011 \text{ e.m.u. cm}^{-3}$ to see if such uniformity could reproduce the observed magnetic anomalies. As far as general widths and amplitudes were concerned, agreement between model and observation was quite good; and some specific anomalies were particularly well matched. In such cases it follows that the relevant near-bottom anomalies may be accounted for solely in terms of the shape of the basement profile and variations in the magnetometer depth. But in many cases specific anomalies were reproduced poorly by the uniform model, notwithstanding its topography. For such anomalies good agreement between model and observation could only be obtained by allowing magnetisation to vary along the profile between extremes of 0.0048 and $0.0257 \text{ e.m.u. cm}^{-3}$.

In summary, then, it would seem that however valid were the conflicting conclusions of Luyendyk and Atwater and Mudie in their respective geographic contexts, neither party is completely correct in general. Moreover, it is now clear that Larson and Spiess were not entirely correct in the specific case of the East Pacific Rise in attributing small scale magnetic anomalies solely to lateral variations of magnetisation. Near-bottom anomalies apparently have no unique cause; in any given instance it may be necessary to invoke both topographic and magnetic effects.

Antitumour immunity

from A. J. S. Davies

THE antagonists of the notion of an immunological antitumour defence mechanism very properly ask why the process involved seems so often to fail. The explanations are increasingly ingenious. Initially, antibody was thought to block the response of hostile lymphocytes to the antigenic tumour; then antigen-antibody complexes and finally antigen have become popular as the blocking factor. It is not too difficult to envisage that antibody would mask those sites on the tumour cell which would otherwise act as recognition foci for lymphocytes. The antibody component of an antigen-antibody complex could do the same as long as it has some free binding sites (that is, the

complexes are in the presence of an excess of antibody). Antigen either free or complexes in antigen excess could presumably combine with any lymphocytes capable of reacting against the malignant cells, thus frustrating their attack.

Overall there is the possibility that some central paralysis of the immune response arises which does not operate by one of the mechanisms already considered. This last possibility is not deemed likely as in many of the instances in which a tumour is growing it is possible to detect by *in vitro* methods the existence of cytotoxic cells and antibodies which have some degree of specificity for the relevant tumour. There is a variety of other explanations which involve non-specific suppression of immunological responses particularly in terminal cases as a consequence of the poor physical condition of the tumour-bearing host.

Hattler and Soehnlen have illustrated the operation of one of these mechanisms (*Science*, **184**, 1374; 1974). These investigators cultured the blood lymphocytes of cancer (usually squamous cell carcinoma of the lung) patients together with their irradiated tumour cells and measured the response of the lymphocyte populations by their capacity to incorporate tritiated thymidine some days later. The response was in all instances much improved by washing the lymphocytes five times before they were allowed to make contact with the tumour target cells. This result is reminiscent of those of Curry and Basham (*Br. J. Cancer*, **26**, 427; 1972) who found that in melanoma patients it was difficult to detect the cytotoxicity of lymphocytes against their specific target tumours unless the lymphocytes were washed six times before the *in vitro* test.

The cell wash eluates of Hattler and Soehnlen effectively inhibited the performance of the cells washed five times but failed to influence the (poor) response of cells washed only once. Fractionation of the eluates yielded a low and a high molecular weight entity neither of which was a particularly effective inhibitor but which in combination did inhibit the cells washed five times. The authors suggest that their results are compatible with the notion that the lymphocytes of the cancer patients may be blocked by antigen-antibody complexes. *In vitro* these complexes can be eluted from the lymphocytes permitting the demonstration of specific antitumour responsiveness. The further exercise of fractionation and the demonstration of two component inhibitors certainly seem to support this idea.

The work of Curry (*Br. J. Cancer*, **28**, 153; 1973) again amplifies the concept. In extension of his earlier studies,

Curry found that the serum of patients with extensive malignant disease contained a blocking factor of low molecular weight which had affinity for lymphocytes but not for tumour target cells. In the serum of patients with less extensive tumours the blocking factor was of large molecular weight and had affinity for both lymphocytes and tumour cells. Curry supposed that this smaller blocking material was antigen and the larger antigen-antibody complexes.

These kinds of studies are extremely valuable in pointing to the complexity of antitumour immunity. A complexity which, without considerable luck, must be mastered before effective and rational immunotherapeutic procedures can be adopted.

Alpha A and beta S

from a Correspondent

INTEREST in the detailed mechanism of the aggregation of deoxy sickle-cell haemoglobin (Hb-S) continues to stimulate the application of new experimental approaches. Wilson, Luzzana, Penniston and Johnson (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 1260; 1974) have used the quasi-elastic light scattering method to study the pregelation aggregation of deoxy Hb-S, and obtain results consistent with a linear association between aggregates. This scheme implies that deoxy Hb-S molecules are bifunctional, with respect to aggregation, and interact in a non cooperative manner, rather than by a concerted scheme in which monomers rather than aggregates are in equilibrium with the gel. Both these alternatives have been considered in previous discussions of the sickling process.

Benesch, Benesch and Yung (*ibid.*, 1504) have exploited their earlier success in the specific pyridoxylation of either the α or the β chains of normal adult haemoglobin (Hb-A) to show that substitution of the N-terminal groups of Hb-S inhibits gelation and increases the solubility of the deoxy form. Modification of the α chains has much more effect than that of the β chains, to an extent equal to that produced by dilution of Hb-S with an equal amount of Hb-A. If this effect could be achieved in the intact red cell, it would be equivalent to reducing the sickling tendency from the degree found in homozygous sickle-cell anaemia to the lesser extent experienced in the heterozygous trait condition. Surprisingly the α -chain modified form of Hb-S obtained by using pyridoxal 5'-phosphate, viz. $(\alpha^{\text{PLS}})_2\beta_2^{\text{S}}$ scarcely differs from Hb-S itself in its oxygenation parameters, and the decreased gelling tendency must, therefore, be caused

by changes in the structure of the deoxy form *per se*.

The same strategy of selective irreversible pyridoxylation of α or β chains has been used by Suzuki, Benesch and Benesch (*Biochem. biophys. Acta*, **351**, 442; 1974) to demonstrate that the Bohr effect in Hb-A is reduced in both α -chain and β -chain pyridoxylated Hb-A, but to a greater extent in the species with modified α chains. It is known from previous work that about one-half of the oxygenation-linked proton exchange of the Bohr effect is associated with the C-terminal β -146 histidines, and about one-quarter with the N-terminal amino groups of the α chains. Elimination of the ionisable protons of these α -chain N-terminal groups by pyridoxylation can account for the observed diminution of the Bohr effect. For the β -chain modified species there is no elimination of an existing Bohr proton binding site, so the change is probably caused by the introduction of a new ionising group whose acid strength decreases with oxygenation, possibly the 5'-phosphate of the attached pyridoxal phosphate reagent used to prepare the β -chain modified species, and which is presumed to form a salt bridge with β -82 lysine in the deoxy but not in the oxy conformation. On oxygenation this residue would therefore bind protons, accounting for the observed decrease in the Bohr effect. These two papers provide an excellent example of the way in which specifically modified but still functional haemoglobin species provide powerful tools for elucidating details of structure-function relationships.

Finally a paper concerned with the red cell itself, and of particular interest in that it deals with the study of individual red cells, always an approach that appeals to biologists and haematologists, who are concerned with red cell populations rather than haemolysates prepared from millions of cells. Brunori, Giardina and Antonini (*J. molec. Biol.*, **86**, 165; 1974) have studied this distribution of the three major haemoglobin parts of trout (*Salmo gairdneri*) blood by single-cell spectroscopy. They find that the erythrocytes contain the component (Hb-IV) which is characterised by the Root effect in the same proportion as in a haemolysate, and which is therefore uniformly distributed. The Root effect (*Biol. Bull.*, **61**, 427; 1931) refers to a very marked dependence of the oxygen binding curve on pH, such that below pH 7 the haemoglobin is only partially saturated in air. It is characteristic of teleost fish haemoglobins, and related in some way to the mechanism of gas secretion into the swim bladder. Brunori *et al.* speculate that the synthesis of several different normal

haemoglobin components in definite proportions in all erythrocytes may be required to maintain a functional situation dependent on a balance of properties of the various components.

J-dependence in nuclear reactions

from P. E. Hodgson

THE general character of the differential cross sections of direct one-nucleon transfer reactions is dominated by the orbital angular momentum L of the transferred particle. Except at low energies when the angular distributions are backward-peaked and similar for all L values, the angular distributions are peaked at an angle in the forward hemisphere that increases with the value of L . In most cases a comparison between the experimental data and distorted wave calculations enables the value of L to be determined.

Since the transferred nucleon also has a spin of $\frac{1}{2}$, the total transferred angular momentum J is the vector sum of L and $\frac{1}{2}$, and since these can be added or subtracted, $J=L\pm\frac{1}{2}$. If the target nucleus has zero spin, this J is just the spin of the final state of the residual nucleus.

The angular distributions of reactions of the same L but different J are usually very similar, but in some cases small but systematic differences have been found. These are called the J -dependent effects, and have proved quite useful in assigning spins to nuclear states simply by the shape of the angular distribution, without any detailed calculations.

These J -dependent effects depend on the transferred orbital angular momentum L and on the incident energy, and are more marked in some cases than in others, so it is clearly important to understand how they arise, both for their intrinsic interest and in order to enhance their utility in nuclear spectroscopy.

The most marked J -dependent effects are found for $L=1$ transitions, and Robson has shown that these can be accounted for very well by distorted wave calculations with a spin-orbit interaction in the incoming and outgoing channels, provided the optical potentials are chosen to fit both the differential cross section and the polarisation of the corresponding elastic scattering.

For $L=2$ and 3 the J -dependent effects cannot be accounted for in this way and several attempts have been made to develop the distorted wave theory, in particular by including the deuteron D-state. This is a complicated calculation, and some of the results show J -dependent effects that are similar to those observed. The difficulty of the calculations has however deterred

an extensive study, so most of the J -dependent effects remain unexplained.

Quite recently a new explanation of J -dependent effects has been proposed: they are attributed to the contributions of two-step processes to the reaction amplitude. The importance of two-step processes is now widely known, especially when the nuclei are deformed and when the direct one-step transition is forbidden or inhibited by some selection rule. A substantial proportion of the reaction can proceed by the incident particle first exciting the target nucleus to a low-lying collective state by inelastic scattering, followed by the particle transfer to the final state. Similarly, the particle transfer can take place first to the ground or to an excited state of the residual nucleus, which can then be excited to the final state by an inelastic process as the outgoing particle leaves. All possible processes of this type combine to give the observed cross section.

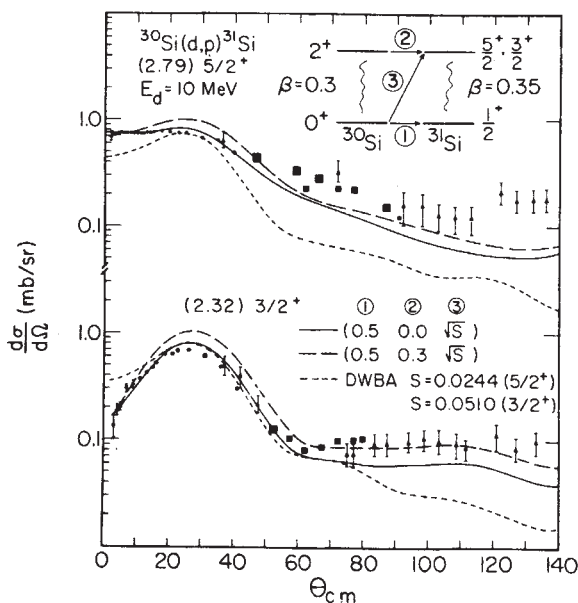
In recent years the formalism for calculating such processes has been extensively developed, and many studies have shown the importance of including two-step processes. It has now been applied by Hoffmann, Udagawa, Coker, McIntyre and Mahlab (*Phys. Lett.*, **50B**, 249; 1974) to study the J -dependent effects in the reaction $^{30}\text{Si}(\text{d},\text{p})^{31}\text{Si}$ at 10 MeV to the 2.32 MeV ($3/2^+$) and 2.79 MeV ($5/2^+$) states of the residual nucleus. As the figure shows, the cross sections of these two reactions differ markedly in the forward direction, although they are both $L=1$ and very similar in energy. The short-dashed curves are standard DWBA calculation, which gives very similar angular distributions for the two reactions, and does not account at all for the difference between them. The calculation including the two-step process via the

$1/2^+$ state of ^{31}Si at 0.75 MeV gives the full curves, which are in excellent accord with the data in the forward direction. The small differences in the backward hemisphere are probably attributable to compound nucleus contributions. A final calculation including those two-step processes through the lowest 2^+ state of ^{30}Si gave the long-dashed curves which do not agree with the data, which is expected since the excited states of the two states of ^{31}Si have a small parentage in terms of the 2^+ state in ^{30}Si , so that this process does not contribute significantly to the reaction.

Inclusion of the two-step contribution to this reaction is thus able to give a very good account of all the J -dependence of the cross sections to the $3/2^+$ and $5/2^+$ states of ^{31}Si . Other effects, such as those caused by the deuteron D-state, may contribute, but in this case at least they are small.

Further calculations by Coker, Udagawa and Hoffmann have shown that the two-step process is also able to account for the $L=2$ J -dependent effects in the $^{28}\text{Si}(\text{d},\text{p})^{29}\text{Si}$ reaction at 10, 13 and 18 MeV to several final states.

This work shows that two-step processes via low-lying collective excitations account for some of the J -dependent effects in one-nucleon transfer reactions in deformed nuclei. The J -dependent effects, however, are also found for nuclei to which this explanation cannot apply. It is therefore necessary to carry out a series of analyses for a range of nuclei and incident energies for different L -values with consistent parameters for the distorting potentials. This will make it possible to establish in a systematic way the contribution of two-step processes to J -dependent effects in nuclear reactions.



Differential cross sections for the one-nucleon transfer reaction $^{30}\text{Si}(\text{d},\text{p})^{31}\text{Si}$ at 10 MeV to the 2.32 MeV $3/2^+$ and 2.79 MeV $5/2^+$ states of ^{31}Si compared with distorted wave calculations. The short-dashed curves are the simple one-step calculation and the full curves show the effect of including the two-step process (1). The long-dash curves show the effect of including the coupling (2) between the excited states, which does not contribute significantly. The figures in the brackets are the spectroscopic factors for the various transitions.

Deterioration of high school students' attitudes to physics

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Australian students taking a physics course based on PSSC displayed a sharp decline in enjoyment of physics. Pupil personality and teacher behaviour variables affected the extent of this decline.

SCIENCE teachers are still concerned about the attractiveness of physics courses. In 1971, in Victoria, Australia, high school physics enrolments showed an absolute decline for the first time in recent history, and a further decline occurred in 1972. In Britain, 2,400 university science places were unfilled in 1973–74, and a complete halt in all university science building programmes for a decade has been predicted¹. Hopes for large increases in the proportion of girls taking physical sciences² have not materialised. There have been attempts to explain this in terms of the poor 'image' of physics and physicists³, a modern distaste for science and technology⁴, the failure of physics curricula to include social aspects of science which are of interest to adolescents, particularly girls⁵, and the operation of economic forces outside the control of the science teacher⁶.

The classroom and the people within it, however, probably remain the central arena of interest for the science education researcher seeking to explain attitudes to science and enrolments in science subjects. There have been many searches for relationships between attitudes or enrolments and other pupil and teacher variables. In most of these studies pupil or teacher variables (but not both simultaneously) have been correlated with pupil outcomes. Although such studies are often interesting they do not permit investigations of the complex interactions which may exist between pupil and teacher variables.

Physics questionnaire research study

The physics questionnaire research study (PQRS) project was set up to investigate the relationships between pupil personality, teacher behaviour and pupils' attitudes to physics. The study was done in Victoria, Australia on Grade 11 pupils taking the first year of a 4 yr course based on the United States PSSC materials. To exert a moderate amount of control over a number of extraneous but potentially influential variables, such as school facilities and home background, the sample was restricted to pupils in coeducational State high schools in the more affluent areas of Melbourne.

Three instruments were used in the project: the physics attitude index (PAI) was employed as a pretest and 8 months later as a post-test; the personal preference index (PPI) and the physics classroom index (PCI) were given as mid-tests. A total of 1,014 students (798 boys, 216 girls) in 58 classes in 34 schools were studied.

The PAI is a 40-item Likert-type scale, yielding scores on four attitudes: first, towards unauthoritarian modes of learning; second, towards physics as an open, flexible, dynamic discipline; third, towards scientists, and fourth, per-

sonal enjoyment of physics. The pretest and post-test means⁶ reveal a favourable initial attitude towards active, participatory modes of learning, an attitude which was maintained during the year. The students also entered the course with the view that physics is an open discipline; at the end of the Grade 11 year, their attitude was less open, the difference being statistically significant, although rather small. The students also held a favourable view of scientists as fairly normal people; again, there was a significant although small decline during the year. The most striking finding, however, was on the fourth scale: although on entry to the course, students expressed a high level of enjoyment in learning physics, eight months later their attitude had declined sharply. Since most science educators would want students to increase, or at least maintain, their interest in a subject, this decline in enjoyment is disturbing.

From the PCI and PPI data some inferences may be drawn about pupil and teacher variables which affect this decline. The theoretical framework underlying the two instruments is the needs-press model of Murray⁷ and Stern⁸. The model indicates that human behaviour may be understood in terms of an interaction between aspects of personality ('needs') and relevant aspects of the social environment ('press'). The PPI contains eight needs scales, taken from Stern's Activities Index: achievement; conjunctivity; deference; play; understanding; order; nurturance and energy. The PCI was devised especially for the PQRS project, and contains eight press scales which correspond to these needs: competitiveness; organisation; compliance; pleasure; intellectualisation; compulsiveness; warmth and stimulation.

A 4×4 analysis of covariance design, with an unweighted means adjustment for unequal cell frequencies, was devised to analyse the data. The analysis of the effects of competitiveness and achievement on enjoyment illustrate the design. Class mean scores on the PCI scale were used to divide the 58 classes into four approximately equal quartile groups, each containing either 14 or 15 classes: classes with teachers regarded as very high, high, low, or very low on competitiveness. Individual scores on the corresponding PPI scale were then used to divide the 1,014 students into four approximately equal quartile groups: very high, high, low, or very low on achievement. The covariance design permitted inferences to be drawn about the effects of these pupil and teacher variables on pupils' post-test attitudes, over and above any effects which could be ascribed to attitudes already present at the start of the course. This type of analysis was carried out 32 times to study the effects of eight needs-press combinations on each of the four attitude variables.

Findings

Of the 64 possible main effects, 25 were significant beyond the $P=0.05$ level, and of these 18 were significant beyond the $P=0.01$ level. Some further details of the findings may be found elsewhere⁹. The teachers' behaviour was found to have little effect on pupils' attitudes to nonauthoritarian

modes of learning. There was a weak inverted U-shaped relationship between attitude scores and teacher compulsiveness (that is, the tendency to organise meticulously the physical environment of the classroom.) Three personality variables were found to be significant: students who are motivated by achievement, serious (low on play) and intellectual (high on understanding) display more favourable attitudes. Factor analysis reveals that these personality variables all lie on one factor. Warm and outgoing students (high on nurturance) also display a more favourable attitude: 'discovery' learning, as it is organised in typical schools, generally requires cooperation with other people, and it is perhaps not surprising that students who are personally more outgoing should enjoy it more than students who are less interested in other people.

Students' views concerning the openness of physics were significantly related to only a small number of the predictor variables. Pupils motivated by achievement tend to hold a more open view, but achievement-pressing teachers (high on competitiveness) tend to promote a more closed view. Apparently, teachers who place heavy stress on achievement, success and examination performance are less likely to maintain the highly open attitudes that most of their students have on entering the course. The only other highly significant finding was the positive relationship between nurturance and openness: apparently, warmth towards other people and receptivity to new ideas are related qualities.

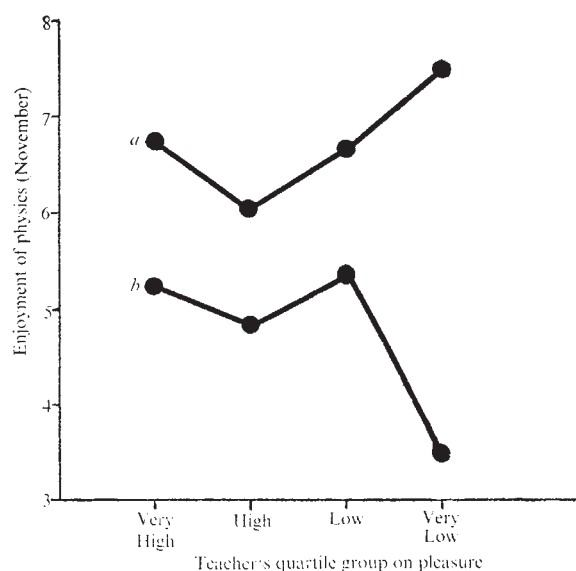


Fig. 1. Enjoyment of physics at end of year by serious and playful pupils under four levels of teacher behaviour. *a*, 'Serious' pupils (below median on play); *b*, 'playful' pupils (above median on play).

Students' attitudes towards scientists were strongly related to only two of the predictor variables: deference and nurturance (factor analysis indicates that these two personality variables lie on a single factor). Thus, pupils who are warm and friendly, and who are more likely to be submissive and conformist, are more likely to regard scientists with affection and tolerance. The relationship is sex-linked: girls significantly outscore boys on the two personality variables and on the attitude scale.

The enjoyment of physics scale displays the most significant findings, and nearly all the predictor variables are involved. In general, intellectually intense pupils—those who are serious, intellectual and motivated by achievement—and pupils who are warm and deferent tend to enjoy

physics more. Intellectually stimulating teachers—those who are intellectual, cognitively well-organised (high on conjunctivity), stimulating and achievement-pressing, and whose classrooms are physically well-organised—tend to be associated with greater enjoyment. Analysis shows that students who are intellectually intense with teachers who are intellectually stimulating display a highly favourable initial attitude, and this is maintained during the year. This is a rather small group: about 6% of the sample (the top quartile group of students in the top quartile group of teachers). Other students with other teachers display a greater or lesser decline. If maintenance of enjoyment is the criterion, the course is effective for a very small group of pupils.

The effects of teacher competitiveness are interesting for two reasons: first, because here is a teacher characteristic which tends to promote one desirable objective (enjoyment) while at the same time hindering slightly the attainment of another (openness); second, because there is an intriguing interaction effect with the corresponding pupil personality variable. Details are given elsewhere¹⁰; briefly, highly achievement-pressing teachers exert a beneficial influence on the enjoyment of highly achievement-motivated pupils, but a relatively deleterious effect on the enjoyment of pupils who are very low in achievement motivation.

Teacher pleasure and pupil play also exert interactive effects on enjoyment. As can be seen from Fig. 1, 'serious' pupils (those below the median on play) enjoy physics more than 'playful pupils. Teachers in the top three quartiles on pleasure have no influence on enjoyment, but teachers in the lowest quartile—very serious teachers—exert diametrically opposing effects upon the enjoyment of serious and playful pupils. Had only class means been considered in this study, no relationship between teacher pleasure and enjoyment would have been found: the correlation between the class means was near zero.

Implications

The PAI has been used in earlier research to compare the outcome of different curricula in other Australian states¹¹. The magnitudes of the teacher behaviour and pupil personality effects found in the PQRS project are much larger than the effects associated with different curricula. Although teacher behaviour is probably more difficult to change than instructional material, the PQRS findings suggest that science educators who wish to bring about improvements in pupil's enjoyment of science subjects ought to concentrate more heavily upon teacher education. The current decline in support for nationally funded science curricula, and the concomitant growth of interest in the pre-service and in-service education of teachers reflect shifts of emphasis which seem to be appropriate.

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Palindromic base sequences and replication of eukaryote chromosome ends

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A new theory is proposed to explain the synthesis of the 5' ends of linear DNA molecules. It suggests that chromosome ends consist of palindromic base sequences. These can form self-complementary hairpin loops, which can be converted by DNA ligase, a specific endonuclease, and DNA polymerase, into completely replicated ends.

DURING DNA replication, new DNA is made by DNA polymerases which add new nucleotides to the 3OH end of growing polynucleotide chains. All known DNA polymerases are able only to extend existing polynucleotide chains and cannot start new ones. For this reason, the mechanism of initiation of new polynucleotide chains has for many years been a fundamental problem in the study of DNA replication¹. There is now good evidence that prokaryotes, eukaryotes and viruses all use a short RNA molecule as a primer for the initiation of new DNA molecules^{2,3}; this RNA primer is probably subsequently removed by nucleases³ before the completion of the daughter molecule by DNA polymerases and DNA ligase. The discovery that the transiently-formed, short pieces of DNA known as 'Okazaki pieces' have a short stretch of RNA⁴ at their 5' phosphate ends, strongly supports Okazaki's model⁵ for the discontinuous synthesis of DNA. This model, in which 'Okazaki pieces' are subsequently joined by DNA ligase to form a complete daughter strand, can now (in conjunction with the idea of RNA primers) provide a satisfactory explanation for the mechanism of replication of circular DNA molecules, though details, especially of the control mechanisms, remain to be filled in.

For linear DNA molecules, however, a fundamental problem remains. Watson⁶ has pointed out that when a linear virus chromosome replicates, the excision of the RNA primers from the 5' ends of the two daughter strands will leave a gap which cannot be filled in by DNA polymerase because there is no adjacent 3'OH end to serve as a template (Fig. 1a). Clearly this problem does not arise in the case of a circular chromosome which has no ends. This may be the reason why bacterial and viral chromosomes are often circular: linear chromosomes require a special mechanism for the replication of their ends. Watson suggested two such mechanisms. Circularisation as occurs in λ phage of *Escherichia coli*, and concatemerisation as in phage T7. Both mechanisms require identical sequences at the two ends of the molecules (terminal redundancy). Watson postulated that all linear DNA molecules must be terminally redundant and must replicate either by circular or concatemeric replicative intermediates.

Here I propose a third mechanism for the replication of the ends of linear DNA molecules which allows them to replicate as linear molecules and does not necessarily require terminal redundancy, circular intermediates, or concatemers. I argue that this third mechanism is more likely to apply to eukaryote chromosomes, which are probably essentially single DNA molecules, than either of

Watson's mechanisms. My theory has the added advantage that it can explain several long known, but hitherto unexplained, properties of the ends (telomeres) of eukaryote chromosomes. As it does not necessarily require terminal redundancy and circular or concatemeric intermediates, it may also be applicable to virus chromosomes, such as chick embryo lethal orphan virus⁷, which seem to lack these properties.

Replication of eukaryote chromosome ends

There is increasing evidence that each eukaryote chromosome consists of a single DNA molecule⁸⁻¹¹. Each chromosome contains many tandemly arranged units of replication (replicons)¹², and each replicon is comparable to the whole chromosome of a bacterium or a virus in that its replication is usually bidirectional. The simplest assumption compatible with the genetic evidence for linear linkage groups is that each chromosome is a linear, rather than a circular, DNA molecule. This means that exactly the same problem of completing the 5' ends of daughter strands will arise as in linear virus chromosomes. The basic problem in applying either of Watson's two suggestions for viruses directly to the replication of eukaryote chromosomes is that, if eukaryote chromosomes consist of single DNA molecules, these will often be many thousands of times longer than those of viruses. Because of the consequent considerable reduction in the effective 'concentration' of free ends, the chances of their ever meeting and pairing, whether to form circles or concatemers will be drastically reduced. The mechanism proposed here overcomes this problem.

The central assumption of my theory is that the nucleotide sequences at the ends of eukaryote linear DNA molecules are palindromic, that is if one always reads with the same polarity, the sequence of bases in one strand is

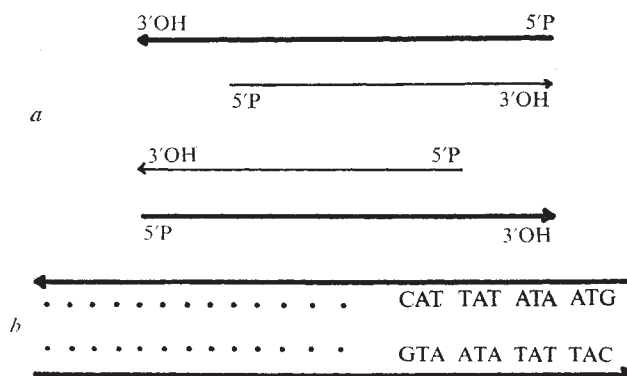


Fig. 1 *a*, The two daughter molecules which result from the replication of a linear DNA molecule, showing the gaps at the 5' end of the newly synthesised strands left by the removal of the RNA primers (the 3' end of a polynucleotide is shown by an arrow, newly synthesised DNA by a thin line). *b*, A terminal palindromic sequence. The sequence on each strand is the same when read with the same polarity.

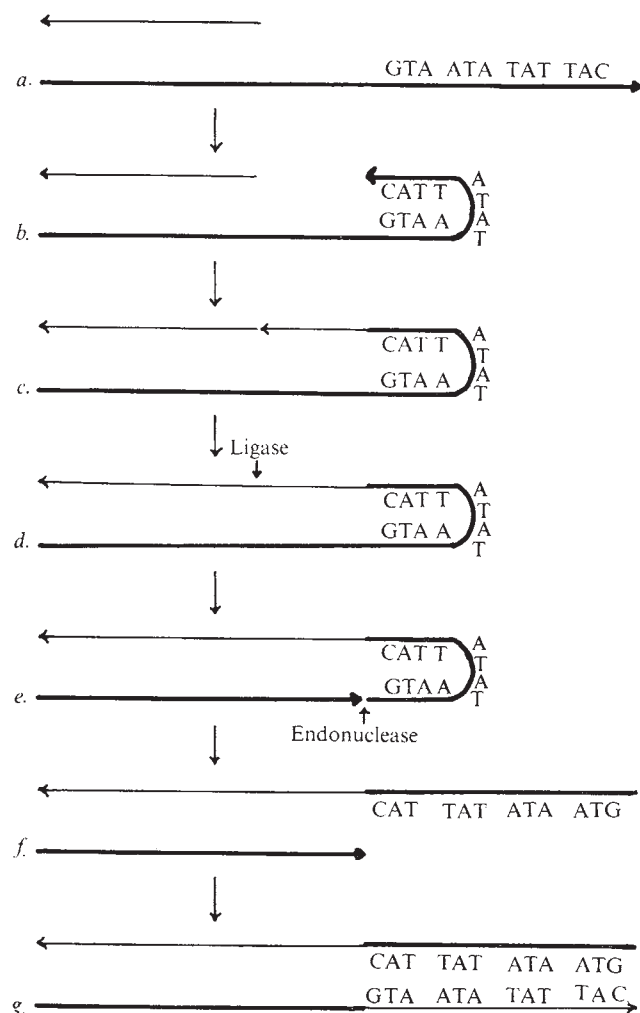


Fig. 2 Model for the synthesis of 5' ends of a daughter DNA molecule (the 3' end of a polynucleotide shown by an arrow, newly synthesised DNA by a thin line). *a*, Gap at 5' end of new DNA; *b*, The self complementary 3' terminal strand of the palindromic sequence base pairs to form a hairpin loop; *c*, 3' OH end serves as a primer for a DNA polymerase to fill in the gap. *d*, DNA ligase seals the remaining nick; *e*, A sequence specific endonuclease nicks the old strand; *f*, The loop unfolds; *g*, DNA polymerase completes the old strand with new DNA. Note this effectively results in the exchange of new and old DNA at one end of every chromosome.

the same as that on the other (Fig. 1*b*), so that the terminal region as a whole has twofold rotational symmetry.

Because of this symmetry the first base in the sequence on one strand is complementary to the last base on the same strand, the second base is complementary to the last but one and so on. This means that the unreplicated single stranded 3'OH end of a daughter chromosome can fold back on itself to form a terminal base-paired loop (Fig. 2*b*).

I suggest that this is the first stage in the replication of the end of the molecule. Subsequent steps are: the closing of any remaining gap by DNA polymerase (Fig. 2*c*); the sealing of the remaining nick by DNA ligase (Fig. 2*d*); the cleavage of the opposite strand by a sequence specific endonuclease (Fig. 2*e*) (this provides a new 3'OH end which serves as a primer for the final step); the unpairing of the loop to provide a template for the final step (Fig. 2*f*); and finally, the completion of the remaining strand by DNA polymerase (Fig. 2*g*).

The mechanism has only two special requirements: a palindromic terminal sequence, and an endonuclease which can recognise one end of this sequence and cleave the correct strand. Both assumptions should eventually be able

to be tested. Palindromic sequences and endonucleases which recognise them are well established in the restriction mechanisms of bacteria¹³. Perhaps they were ancestral to the mechanism postulated here. The palindrome need not be perfect since there must be at least two bases at the end of the loop which cannot pair and therefore need not be complementary.

Telomeres of eukaryote chromosomes

Although eukaryote chromosomes probably consist of a single DNA molecule, very little is known of the way this is folded in mitotic and meiotic chromosomes. In particular, it is not known whether the two ends of the molecule lie at the ends (telomeres) of the chromosome. In the case of meiotic chromosomes, however, the correlation in maize between the linear sequence of chromomeres and the linear sequence of genes in a linkage group makes it reasonable to assume that this is the case. If, therefore, one assumes that, as a general rule, the ends of the DNA molecule do in fact form part of the telomeres then the existence of terminal palindromic sequences could explain several intriguing properties of the telomeres.

First, there is evidence that broken ends of chromosomes behave differently from the natural ends in that they are 'stickier' and have a strong tendency to rejoin or to become permanently joined to broken ends of other chromosomes¹⁴⁻¹⁶. This is to be expected because broken ends are probably often single stranded, and if they contain partially complementary sequences (for example, one of the widely dispersed repetitive sequences¹⁷) or precisely complementary sequences (as in rejoining) can base pair and be irreversibly joined by polymerase and ligase action. By contrast, telomeres could not become permanently joined because of the sequence-specific endonuclease which would split them apart, if they did happen to pair and become joined when in the single stranded state.

A second property of telomeres is frequent temporary association of the telomeres of homologous chromosomes^{18,19}. Even in the absence of palindromic ends, the corresponding ends of homologous chromosomes could associate by 'side-to-side' base pairing if the appropriate strand of each is incomplete (whether because of exonuclease digestion or the delayed completion of replication, Fig. 3*a*). The existence of terminal palindromic sequences, however, would provide an alternative explanation. 'End-to-end' pairing to form a circular dimer would be possible if the 5' ends of the two homologous chromosomes were partially digested by exonuclease action (Fig. 3*c*). Indeed, this would provide an alternative pathway for the replication of 5' end if gaps in this dimer were repaired by DNA polymerases and ligase and then staggered nicks inserted (Fig. 3*d-e*), and the replication completed as before by DNA polymerase. This pathway, in contrast to the previous one, would be most efficient if the palindrome was perfect, since all nucleotides could take part in base pairing.

The occurrence of pairing between homologous telomeres in either of the two ways suggested here could explain cases of late separation of telomeres in mitosis²⁰.

In meiosis, the telomeres normally form strong end-to-end attachments at the beginning of diakinesis^{15,19,21}. Darlington's theory of the terminal chiasma²¹ postulated that this 'terminal affinity' was quite distinct from the normal lateral pairing of chromosomes during the earlier stages of meiotic prophase. I suggest that terminal affinity could be caused by the end-to-end association of palindromic ends as shown in Fig. 3*d*.

Nonhomologous chromosomes

Although the replication mechanism postulated does not require terminal redundancy, and would work perfectly

well even if the palindromic sequences at the two ends of a chromosome were different, there seems no obvious reason why they need be different. So it would be simplest to assume that the telomeres of all chromosomes in any one eukaryote are identical. The considerable evidence for

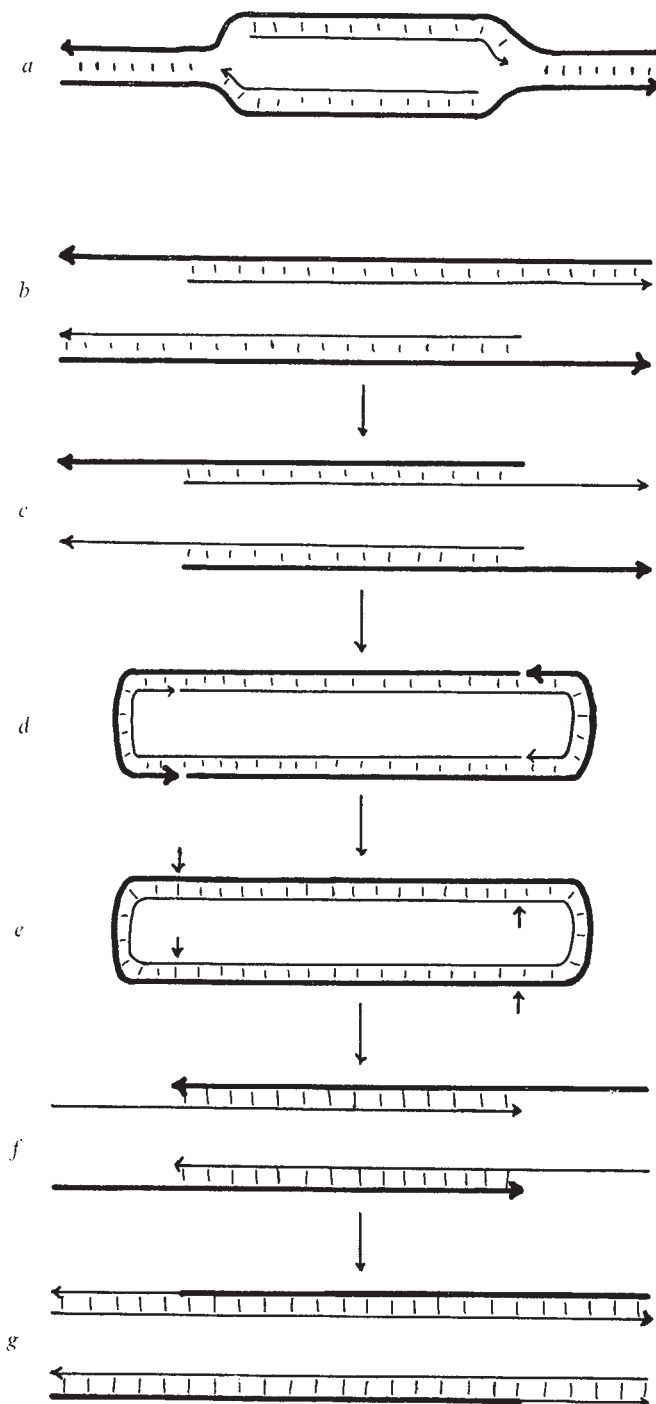


Fig. 3 *a*, Delayed replication provides a simple explanation for the late separation of telomeres at anaphase, which does not require palindrome sequences (the 3' end of a polynucleotide shown by arrow, newly synthesised DNA by a thin line). *b-g*, An alternative model for the replication of 5' ends and the delayed separation of telomeres. *b*, Gaps at 5' ends left by removal of RNA primer; *c*, an exonuclease digests the 5' ends of old DNA; *d*, base pairing between the single-stranded 3' ends of the two homologues; *e*, DNA ligase seals the nicks, and the sequence-specific endonuclease makes new nicks (arrows); *f*, unpairing; *g*, DNA polymerase completes the molecule using the newly exposed 3' ends as primers. Note that in contrast to the mechanism in Fig. 1 the new strand contains no old DNA. Instead an exchange of old DNA has occurred between the two daughters.

direct or indirect attachments between the telomeres of nonhomologous chromosomes¹⁸ lends some support to this idea.

At leptotene of meiotic prophase in many organisms, all the telomeres become gathered together and attached to a small area of the nuclear envelope, presumably so as to facilitate pairing during zygotene^{16,21,23}. This characteristic polarised 'bouquet stage' persists until pachytene when pairing is complete. This phenomenon strongly indicates that all telomeres possess some common structural feature, which might simply be a common palindromic sequence, which could serve not only for replication but for recognition by the apparatus that brings the ends together.

Dupraw¹⁸ has instanced several cases where many or all of the mitotic chromosomes are attached to each other by fine connectives. He even goes so far as to suggest that all the chromosomes of a eukaryote haploid set are simply parts of a single continuous circular DNA molecule, as in bacteria. If this were true it would make the replication mechanism postulated here totally unnecessary. However, Dupraw's model raises more problems than it solves. For example, he fails to discuss the difficult problem of how two haploid super chromosomes could be integrated to make a single diploid circle.

He does recognise, however, that this extreme model can only be compatible with the genetic evidence for the independent assortment of linkage groups if the postulated 'DNA connectives' between the nonhomologous chromosomes are unusually 'labile'. His explanation of this is that the connectives contain no functional genes and that the lability is caused by normal recombination. This would imply, however, that very large amounts of DNA contain no genes but simply serve to connect the chromosomes together. In maize and *Drosophila* such nonfunctional interchromosomal connectives would have to amount to 60% and 75% of the lengths of the functional part of the chromosomes, respectively, if they are to give a recombination frequency of 50%. The need to postulate such huge segments of nonfunctional DNA, whose sole function is to join nonhomologous chromosomes together in such a way as to allow independent assortment, can be totally avoided by supposing instead that the observed linkages are purely temporary and that their lability is not the result of normal genetic recombination.

Indeed, if as suggested here, the ends of all chromosomes have identical sequences, which are sometimes single-stranded, one might expect nonhomologous chromosomes on occasion to join together by base pairing between these cohesive ends. If this did occur, and if DNA ligase were to join the chromosomes covalently, the specific endonuclease would ensure that this joining was not permanent. In principle the chromosomes could associate reversibly into a single superchromosome as indicated in Fig. 4*a*. Though this kind of mechanism could explain some examples of chromosome association, others involve association between regions other than the telomere, and could be explained in this way only if it were assumed that the DNA ends are not at the telomere.

I am grateful to the referee of this paper for drawing my attention to a paper by White²², who suggested that the occurrence of multivalents of terminally associated chromosomes in the meiosis of *F₁* hybrids (and other situations where normal pairing is reduced) could be caused by minute regions of homology at the tips of nonhomologous chromosomes, and not by reciprocal translocation as had hitherto been believed. This hypothesis of reduplicated telomere regions was supported by Callan, who in a footnote to White's paper said "all new telomeres are probably homologous". Despite criticisms¹⁹ it remains a reasonable interpretation of at least some of the data, which is fully consistent with my assumption that all telomeres in any one species are homologous.

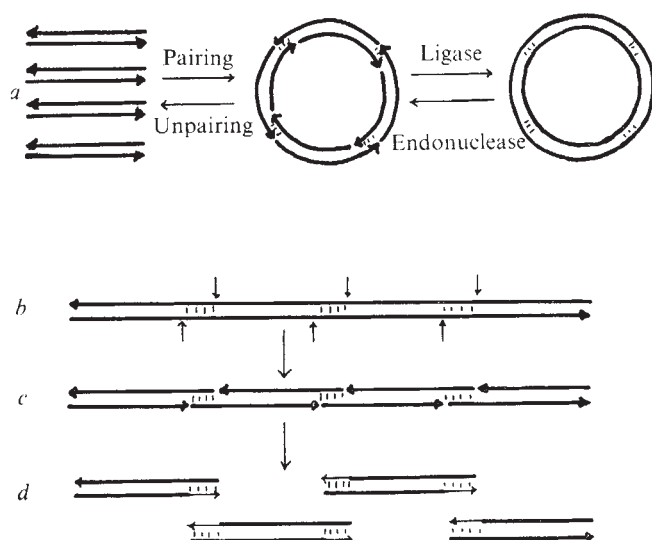


Fig. 4 *a*, The reversible formation of superchromosomes or, reading from right to left, an hypothesis for the origin of several linear eukaryote chromosomes from a single circular prokaryote chromosome (the 3' end of a polynucleotide shown by arrow, newly synthesised DNA by a thin line). *b-d*, Mechanism for the formation of minichromosomes during chromosome diminution or hypotrich ciliate macronuclear formation; *b*, the small arrows show the sites at which a specific endonuclease makes nicks at each end of special internal palindromic sequences; these sequences differ from the palindromic sequences at the original ends of the chromosomes. *c*, The nicks are made and, *d*, a DNA polymerase extends each newly formed 3'OH end, forming complete palindromic sequences at the new ends, and causing the new mini chromosomes to separate.

White pointed out that if many or most chromosomes have homologous telomeres, multivalent formation must normally be suppressed during meiosis. He suggests, however, that the homologies have no great evolutionary importance and originate quite fortuitously (though very commonly) by the reciprocal translocation of extremely minute segments, and the survival of resulting deficiency-duplication homozygotes. If the homologies are mere accidents, there is no obvious reason why the evolution of a general mechanism to suppress multivalents should have been selectively favoured over the simple alternative of eliminating the unnecessary duplications. By contrast, my suggestion that telomeres consist of identical palindromic sequences, with an indispensable role in replication, provides a strong selective advantage for the suppression of multivalent formation.

Chromatin diminution

In eukaryotes there are at least two instances where normal chromosomes are cleaved into smaller linear pieces or 'minichromosomes' which are subsequently replicated; these are chromatin diminution in the somatic cells of the worm *Ascaris* and other animals²³, and macronuclear formation in the ciliates *Stylonychia* and *Euplotes*^{24,25}. The replication of these 'new ends' will present exactly the same problems as that of the original ends of the normal chromosomes. I suggest, therefore, that these new ends must also consist of palindromic sequences, and that they are replicated in essentially the same way as the normal telomeres. Moreover, the site specific nuclease which functions in the replication of minichromosomes could be the same enzyme as the one which initially cuts the chromosome into minichromosomes, as both have to cut at the same site in the sequence. The only difference is that in replication only one strand is cut (Fig. 2e) whereas in making minichromosomes two staggered cuts are needed on opposite strands

(Fig. 4b). Two single-stranded ends will be produced and when the other strand is completed by DNA polymerase each minichromosome will have a complete palindromic end. To ensure that this cleavage does not occur in the germ line of *Ascaris* and in the micronuclei of the ciliate I suggest that the palindromic sequence and the endonuclease involved are different from those at the normal chromosome ends, and that the synthesis of the endonuclease is normally repressed and has to be derepressed to initiate chromatin elimination. This special endonuclease and palindromic sequence may have evolved from the normal ones involved in telomere replication.

Origin of eukaryote chromosomes

A necessary and crucial step in the evolution of a group of linear eukaryote chromosomes from a single circular bacterial chromosome must have been the evolution of some special mechanism for the replication of the chromosome ends. Unless this mechanism was present right at the outset, pieces would have been lost from the ends of the chromosome at each replication. Therefore I suggest not only that the palindromic telomere mechanism evolved from a bacterial restriction mechanism, but that this change was all that was needed to convert a circular chromosome into several linear ones. Thus Fig. 4a if read from right to left could be taken to represent the origin of separate eukaryote chromosomes.

It should be possible to test all the predictions of my model as methods for sequencing DNA and for isolating and working with unbroken eukaryote DNA improve.

Note added in proof: Work recently published by Wilson and Thomas (*J. molec. Biol.*, **84**, 115-144 (1974)) reveals numerous nearly perfect palindromes in eukaryote DNA, but does not indicate whether any are terminally located as my model would predict.

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letters to nature

X-ray observations of the Coma and Virgo clusters from Copernicus

THE X-ray sources in both the Virgo and Coma clusters of galaxies are known to be extended. We report here observations to determine the spatial characteristics of each source using the two grazing incidence reflecting telescopes and the collimated proportional counters on board Copernicus¹.

The observations were made on January 6 and 7, 1973, and January 26 and 27, 1973, for the Virgo and Coma clusters respectively. In both cases only the widest field of view on the X-ray telescopes was used (effective beam diameter about 12') to observe seven regions, one centred on the source, and six spaced equally 10' from the centre position. The two telescopes covered the energy ranges 1.5 to 4.6 keV (3 to 8 Å) and 0.48 to 1.45 keV (6 to 18 Å) and the collimated counters the range 2.5 to 7.5 keV. A total of 254.7 min of usable data was acquired from the Virgo cluster and 382.6 min from the Coma cluster; the total observation time was approximately equally divided between the seven observing regions.

Coma cluster: It is now well established that the Coma cluster is an extended X-ray source²⁻⁵. The Uhuru results³⁻⁵ were originally analysed in terms of a uniform emitting disk, with a resulting diameter of $36' \pm 4'$ (ref. 5). A reanalysis of the data⁴ by Lea *et al.*⁶ in terms of more realistic models, including an isothermal gas sphere, resulted in smaller source dimensions. For the isothermal sphere models Lea *et al.* deduced a best-fit core radius $a = 16' \pm 3'$, and for a model in which the gas density $\rho \propto r^{-2}$ (the asymptotic approach to an isothermal sphere) they obtained $a = 7' \pm 2'$. Models for which the emission is proportional to the first power of the density, predicted for some nonthermal mechanisms, were excluded by their analysis.

The Uhuru observations could not distinguish between thermal or power-law X-ray spectra, but the low energy results from the rocket flight of Gorenstein *et al.*⁷ show that the spectrum is best fitted by thin source bremsstrahlung with $kT = 8.1 \pm 1.5$ keV (including an energy-dependent Gaunt factor), taking into account the absorption of 2.10^{20} hydrogen atoms cm^{-2} along the line of sight to Coma. A power-law spectrum appears to be excluded by the need for a hydrogen column density lying outside the acceptable range 1.5 to 2.5×10^{20} hydrogen atoms cm^{-2} (for the galactic latitude of Coma).

The X-ray telescopes on Copernicus observed a central region of Coma and six adjacent regions with 12' effective diameter⁸. A total of 63 min observing time was spent on the central region, which contains the cluster kinematic centre, the Uhuru X-ray centroid and the two prominent galaxies NGC4874 and NGC4869. In the 0.48 to 1.45 keV range, the 2σ upper limit on the flux from the central region corresponds to 1.3×10^{-2} photons $\text{cm}^{-2} \text{s}^{-1}$, and in the 1.5 to 4.6 keV band the recorded count, at 3σ above background, corresponds to a flux of 9.4×10^{-3} photons $\text{cm}^{-2} \text{s}^{-1}$, assuming the spectrum described here. In the outer regions, the average signal over the six bins gave 3.3×10^{-3} photons $\text{cm}^{-2} \text{s}^{-1}$ in the higher energy band (a 2.7σ result).

We have compared these observations with the counts predicted by spectral fits to the combined data from Uhuru and Gorenstein *et al.*⁶:

$$dN/dE = (0.044/E) G e^{-E/kT} \text{ photons cm}^{-2} \text{s}^{-1} \text{keV}^{-1}$$

where the Gaunt factor $G = 0.8 (E/kT)^{-0.4}$ $E/kT > 0.1$
and $G = 1 - \log_{10} (E/kT)$ $E/kT < 0.1$
with $kT = 8.1 \pm 1.5$ keV.

For the efficiencies of the telescope system covering the 0.48 to 1.45 keV band, the predicted count from the whole cluster is 375 ± 41 . The 2σ upper limit from the central bin was 54 counts and we can therefore state with 95% confidence that less than 14% of the total emission comes from the central 12' for this energy interval. For the higher energy band (1.5 to 4.6 keV) the predicted count from the whole cluster is 287_{-49}^{+18} . The observed count after background subtraction was 57 ± 27 (at 3.2σ above the mean background level, or $20 \pm 10\%$ of the predicted total).

Assuming an isothermal gas distribution $\rho = \rho_0 (1 + r^2/a^2)^{-3/2}$, the above result leads to a lower limit of 19.5' for the core radius in the 0.48 to 1.45 keV band, and a value $a = 15_{-3}^{+7}$ from the higher energy band. The positive error on the latter result is reduced by noting the ratio between the counts in the outer and central bins, giving finally $a = 15' \pm 3$. This agrees well with the value $a = 16' \pm 3$ from Lea *et al.*⁶ for a similar energy interval.

Following Lea *et al.* we have also fitted the density function $\rho = \rho_0 (1 + r^2/a^2)^{-1}$, or emission $\varepsilon \propto (1 + r^2/a^2)^{-2}$, approached asymptotically by an isothermal sphere. For this function, the upper limit for a becomes 10' in the low energy band, and $a = 8' \pm 2'$ for the higher energy band, the latter measurement again agreeing with $a = 7' \pm 2'$ from ref. 6.

As a test on the reliability of the Copernicus results quoted here, we have compared them with predictions based on the Uhuru and Gorenstein results. The count observed in the 2.5 to 7.5 keV detector, which viewed the whole cluster throughout the observations was $2,873 \pm 192$ counts at 19σ above background. The total predicted count was $2,716 \pm 275$, the agreement to 5% accuracy being well inside the statistical uncertainties involved.

Thermal models for the origin of the Coma cluster X-ray emission are presently divided between those which invoke accretion for the origin of the gas⁹⁻¹¹ and those which explain the intracluster gas as a wind emanating from the cluster centre¹² with the energy coming from violent activity (see ref. 13), or by frictional heating. The inverse Compton models^{14,15} seem to be ruled out by the thermal shape of the X-ray spectrum, supported by the present observations, as well as by difficulties with the emission mechanism itself when applied to Coma^{16,17}.

The temperature distribution in the gas both for the accretion models and the wind models (heating by mechanisms inside galaxies, and by friction) is predicted to fall with angular distance from the cluster centre. The tentative results obtained in the present observations clearly show this trend, whilst not allowing a preference among these individual thermal models to be firmly stated. But the 'heating inside galaxies' model of Yahil and Ostriker¹² is given some support as the dominant mechanism, since the core radii found here agree well with those predicted.

Virgo cluster: Several rocket flights have observed a source at 1 to 10 keV in the direction of M87 and the Virgo cluster, as summarised by Lampton *et al.*¹⁸ for flights before 1971. Since then, the Lockheed Group have observed the

source between 0.2 and 1.6 keV (ref. 19) and found it to consist of a point source within a few arc mins of M87 and an extended source with its centroid displaced $\sim 0.2^\circ$ in the direction of the optical jet. The point source was found to contain $60 \pm 30\%$ of the total low-energy X-ray emission; the Uhuru data are consistent with up to 35% of the emission coming from a point source.

The best fit to the Uhuru spectral data²⁰ is a power law, supposedly arising from the inverse Compton effect. Extrapolation of this spectrum to lower energies, combined with the spectral points of R.C. Catura *et al.* (unpublished results consistent with the upper limits of Gorenstein *et al.*²¹) leads to a necessity for ageing of the electrons in the ambient photon field; self absorption, suggested by Kellogg *et al.*²⁰, is ruled out by the spectral data of R. C. Catura *et al.* (unpublished). Alternatively both sets of data are consistent with a thermal bremsstrahlung from a plasma at 36×10^6 K, with only 1.5×10^{20} hydrogen atoms cm^{-2} along the line of sight, consistent with radio observations in this direction.

A reanalysis by Ricketts²² of previous Leicester rocket flights which observed M87 shows agreement with the above bremsstrahlung spectrum in temperature and normalisation, without strong evidence for any variability (see also ref. 18). Uhuru observed no variability greater than 30% between observations four months apart²⁰.

As with Coma, the Copernicus telescopes were pointed at a central bin and six neighbouring regions of the Virgo cluster, where the central bin contained M87. The counts obtained were compared with those predicted from the thermal spectrum above:

$dN/dE = 0.31/E \exp(-E/3.1) \exp(-\sigma N_H)$ photons $\text{cm}^{-2}\text{s}^{-1}\text{keV}^{-1}$ with $N_H = 2.5 \times 10^{20}$ hydrogen atoms cm^{-2} .

In the 0.48 to 1.45 keV band we observed 71 ± 21 counts in 63 min of observation on the central bin (a signal 4.1σ above background), compared with $1,032 \pm 62$ counts predicted from the whole cluster; only $7 \pm 2\%$ of the predicted count was in the central bin. In the 1.5 to 4.6 keV band, we can set an upper limit of 10% of the predicted total cluster emission being in the central region containing M87 and the jet. These percentages are clearly inconsistent with the $\sim 60\%$ found by Catura *et al.*, and are well below the 35% upper limit set by Uhuru.

Assuming the isothermal gas distribution $\rho = \rho_0 (1 + r^2/a^2)^{-3/2}$, our results imply a core radius $a > 23'$ for the 1.5 to 4.6 keV band, and $a = 28' \pm 4'$ for the 0.48 to 1.45 keV band. Lea *et al.*⁶ found $a = 25' \pm 4'$ for the 2 to 6 keV range.

In the 2.5 to 7.5 keV detector with a wide field of view, the total observed count was $1,934 \pm 153$, compared with $1,711 \pm 174$ from the Lockheed/Uhuru spectrum quoted above. So our observed flux is consistent with Uhuru to an accuracy of $\approx 10\%$. We therefore infer that the total X-ray flux from the Virgo cluster in this energy range did not change by more than 10% between early 1971 and January 1973.

The OAO Copernicus observations of the Coma and Virgo clusters are consistent with the high-temperature isothermal gas sphere models already proposed for the emission, with some indication that the low-energy X-ray emission is more extended, as hypothesised for the Perseus cluster²³. There is no strong galactic X-ray source at the centre of either cluster, in contrast to the bright X-ray emitting Seyfert galaxy NGC 1275 at the centre of the Perseus cluster²³. We note that all three clusters contain active galaxies (strong radio sources) near their centres, but only Perseus has a strong infrared emitting Seyfert galaxy.

The absence of a strong point source in the Virgo cluster is in disagreement with Lockheed rocket flight data. We do find good agreement, however, with Uhuru data from the whole cluster (including M87) in the 2.5 to 7.5 keV range.

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Prediction of radio structure in the two largest redshift QSOs

THE discovery that the optical objects identified with OH471 (0642+449) and OQ172 (1442+101) are QSOs with redshifts of 3.40 (ref. 1) and 3.53 (ref. 2), respectively, has stimulated considerable interest in their optical^{3,4} and radio properties⁵.

Here we present predicted angular sizes for the radio sources based on incoherent electron synchrotron emission. Such angular sizes, combined with a cosmological interpretation of the redshifts, determine the minimum variability timescales consistent with the absence of relativistic effects.

Figure 1 shows the radio spectral data of OH471 and OQ172 (ref. 5) and decompositions of the spectra into canonical self-absorbed synchrotron components. We have used the minimum number of canonical components consistent with the data and have made the simplifying assumption that all components of a given source have the same spectral index, α . Uncertainties in the data preclude a meaningful determination of the spectrum at the highest radio frequencies.

Table 1 lists the resulting properties of each component. The frequency ν_n and the spectral flux $F\nu_n$ connote the intersection of the extrapolated opaque and transparent portions of the spectrum of each component. The $\nu_n \approx 6.5$ GHz and the $\nu_n \approx 18.4$ GHz components of OH471 are not very well established, but any combination of canonical components in this frequency range results in similar angular sizes.

Table 1 Theoretically expected angular radii and timescales

Source		OH471 (0642+449)		OQ172 (1442+101)
z		3.40		3.53
ν_n [GHz]	0.84	6.5	18.4	0.26
F_{ν_n} [Jy]	3.0	0.63	0.83	2.6
	0.89	0.89	0.89	0.63
α				
$\theta(F_V^C = F_V^S)$ [10^{-3} arc s]	3.8	0.28	0.13	6.1
$\theta(u_r = u_m)$ [10^{-3} arc s]	4.9	0.33	0.14	12
$\theta(u_e = u_m)$ [10^{-3} arc s]	5.1 (4.8)*	0.31 (0.29)	0.12 (0.12)	14 (13)
$\theta(u_r = u_e)$ [10^{-3} arc s]	5.1 (4.4)	0.27 (0.23)	0.10 (0.08)	17 (14)
D_1 [Gpc]		20† (55)		21 (59)
$R(F_V^C = F_V^S)$ [pc]	19 (50)	1.4 (4)	0.6 (1.7)	30 (80)
$t_V(F_V^C = F_V^S)$ [yr]	90 (250)	6 (18)	3 (8)	150 (400)
t_V^{-1} [% per yr]	1.1 (0.4)	15 (5)	30 (12)	0.7 (0.24)
$B(u_e = u_m)$ [mgauss]	15 (12)	130 (100)	300 (260)	6 (5)
$U(u_e = u_m)$ [$M\odot c^2$]	50 (600)	1 (20)	1 (15)	100 (1000)
				19 (15)
				30 (300)

*Two values appear for each parameter dependent upon the cosmological model: They are calculated for standard Friedmann cosmologies with $q=1$ and with $q=0$ (in parentheses).

†The luminosity distance is based upon $(c/H)=6$ Gpc ($H=50$ km/s Mpc) ($H=50$ km/s Mpc) $^{-1}$.

The angular size of a compact nonthermal source depends only weakly on assumptions about physical properties or distance, if the radiation is incoherent electron synchrotron emission with no relativistic bulk motions¹¹⁻¹³. Angular sizes and assumed distances therefore imply a minimum timescale of variability in the absence of highly relativistic effects^{14,15}.

1 Jy — 1 f.u.

Estimation of the angular size of a compact radio source with known spectral form rests upon the establishment of its maximum brightness temperature. Compact nonthermal radio sources show a rather narrow range of maximum brightness temperatures, 10^{11} K $< T_n < 10^{12}$ K (refs 6, 7). Although this results partly from observational selection, it also follows as a direct consequence of synchrotron theory^{6,7}. For a given radio

spectrum, the small dispersion in brightness temperature allows an accurate estimate of angular radius θ , since $\theta(\nu_n, F_{\nu_n}) \propto T_n^{-1/2}$. Table 1, which lists angular radii calculated according to assumed values of four different physical source parameters, demonstrates the insensitivity of the angular radius (brightness temperature) to physical conditions in a source. The parameter (F_V^C/F_V^S) represents the ratio of spectral flux in Compton-scattered synchrotron photons to (extrapolated) synchrotron spectral flux. The other three ratios involve the energy densities in relativistic electrons (u_e), magnetic field (u_m), and radiation (u_r).

The insensitivity of θ to each of these parameters follows from readily derivable relationships (see Table 4, ref. 8):

$$\theta(F_V^C/F_V^S) \propto (F_V^C/F_V^S)^{-1/(2(3+2\alpha))} (1+z)^{(2+\alpha)/(3+\alpha)}, \quad (1)$$

$$\theta(u_r/u_m) \propto (u_r/u_m)^{-1/10} (1+z)^{3/5}, \quad (2)$$

$$\theta(u_e/u_m) \propto (u_e/u_m)^{-1/17} (1+z)^{9/17} D_1^{-1/17}, \quad (3)$$

$$\theta(u_e/u_r) \propto (u_e/u_r)^{-1/7} (1+z)^{3/7} D_1^{-1/7}, \quad (4)$$

with D_1 the luminosity distance. So even crude bounds to any of these ratios greatly restrict the angular sizes. In many cases, optical and X-ray observations require $(F_V^C/F_V^S) < 1$ (ref. 8) and the radio data alone generally suggest $(F_V^C/F_V^S) < 1$ (refs. 9, 10). If $u_r > u_m$, Compton energy losses dominate synchrotron losses; thus, to avoid catastrophic luminosities, $(u_r/u_m)^{1/10} \lesssim 1$. Although equipartition ($u_e = u_m$) seems unlikely in evolving compact sources and experience suggests $u_e > u_m$ (refs. 7, 8) it seems reasonable to expect that $(u_e/u_m)^{1/17} \approx 1$. Finally, (u_e/u_r) represents the weighted radiative lifetime of electrons normalized to the travel time of light across the source; consequently, in the absence of noncentral accelerations, $(u_e/u_r) \gtrsim 1$.

The canonical incoherent synchrotron model admits angular sizes smaller than those given in Table 1 only in the presence of relativistic bulk motions (for example, small pitch angles or relativistic expansion)^{8,9}. These minimum angular radii range in the lower frequency components between 2 and 6×10^{-3} arc s, and even the $\nu_n = 18.4$ GHz component of OH471 should have an angular radius in excess of 0.1×10^{-3} arc s. Very long baseline interferometric (VLBI) observations could confirm, therefore, the consistency of the canonical assumptions.

The source angular radius indicates a physical size

$$R = D_1 \theta (1+z)^{-2} \quad (5)$$

and, for variable sources, an index of variability

$$i_v = (R/c t_v) (1+z) = (D_1 \theta / c t_v) (1+z), \quad (6)$$

where the variability timescale $t_v = 1 \text{ d } |F_V/dt|^{-1}$. In the absence of highly relativistic effects, $i_v \lesssim 3$ (ref. 9); conversely, for $i_v \lesssim 3$, there is a minimum t_v consistent with the assumed angular size and distance. Table 1 lists for each source com-

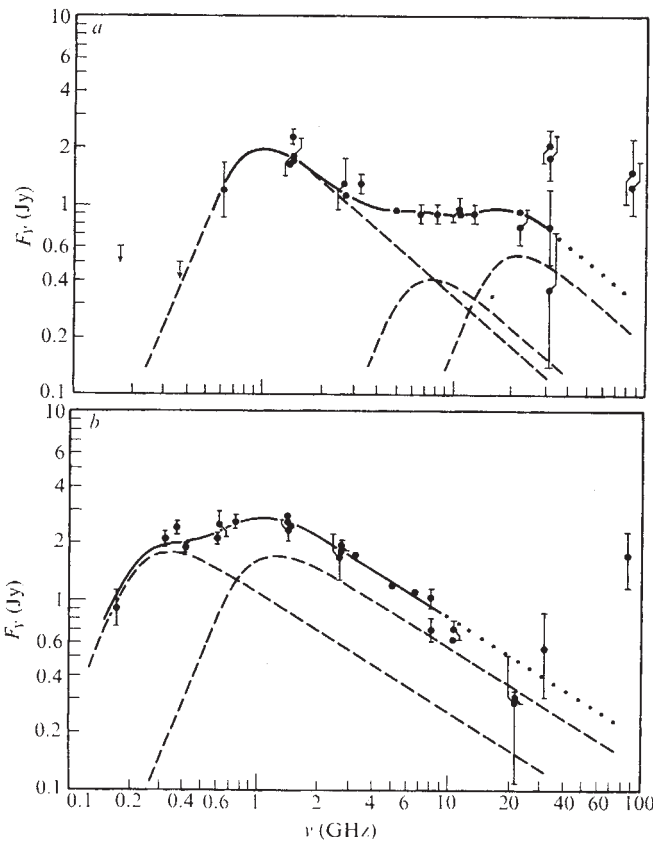


Fig. 1 Radio spectral data of: *a*, OH471 (0642+449); *b*, OQ172 (1442+101) from ref. 5. Points with no error bars have errors within the solid circles. Dashed curves represent best fit decompositions of those portions of the spectra indicated by solid lines into canonical self-absorbed synchrotron components.

ponent values of R and t_v , based on luminosity distances D_l calculated (assuming $c/H=6$ Gpc; $H=50$ km/(s Mpc)) for a Friedmann cosmological model with $q=1$ and for one with $q=0$. We note that for redshifts as large as three, the choice of cosmological model (q) significantly influences the allowed values of t_v and t_v^{-1} as well as some derived parameters such as the equipartition (minimum) energy content.

Present observations do not firmly establish the existence of variability in either of these sources, although Gearhart *et al.*⁵ suggest the possibility of a 20% decrease in OH471 at 1.4 GHz during a 4 yr span, as compared to a value $t_v^{-1} \lesssim 1\%$ per yr given in Table 1 for the relevant component. If such rapid variations are present, VLBI observations could ultimately decide among possible alternatives to the canonical assumptions made here^{8,9}.

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How long do radio galaxies emit at radio wavelengths

MANY clusters of galaxies from Abell's catalogue¹ have recently been observed at 1,400 MHz with the 300-foot radio telescope of the NRAO (ref. 2 and F. Owen, to be published). On the Palomar Sky Survey prints we inspected 355 clusters of galaxies (111 of them were observed by H.M.T.², the remaining 244 by F. Owen, to be published) which belong to distance group 5 and thus are situated at almost the same distance. Radio emission in excess of 0.1 flux units (f.u.) was detected from 95 out of the 355. A radio source was identified with a cluster of galaxies if it was within 5 arc min from the cluster centre, the diameter of which is about 25 arc min.

The study showed that:

(a) There are 27 clusters of galaxies in which the brightest is a cD-type galaxy (cluster type I according to Bautz and Morgan³). Eighteen of them emit at radio frequencies. Since the probability of detecting a radio source at the position of a cluster of galaxies is one in the case of observations of 22 clusters, if objects of both types are distributed randomly², the most probable number of

incorrect identifications is one or two.

(b) There are four clusters in which the brightest galaxy is a compact galaxy and two of them are radio emitters.

(c) Fifty-one clusters have two or three bright members, one of which is either a cD-type or a compact galaxy, or a peculiar galaxy, or a close double galaxy. Radio sources are found at the positions of 39 such clusters. Two or three of the detected sources may be located there by chance.

(d) There are 33 clusters in which a bright elliptical galaxy dominates. Radio emission is detected at the positions of only three clusters and two of these identifications could be by chance.

(e) There are 45 clusters which contain two or three bright E galaxies. Radio sources are found at the positions of six of them and two of these identifications are most probably spurious.

Since the angular resolution of the survey is greater than the diameters of the clusters it is possible to go one step further and check whether the dominant galaxy in the cluster is within the error box of a radio source position. In almost all the above cases this is so. Since radio sources are generally not resolved this means that radio emission originates in one galaxy of the cluster rather than in many members of it. Thus one can with great confidence identify the radio sources in the clusters considered with centrally located, bright cD compact, peculiar, close double or elliptical galaxies. The radio luminosities of these galaxies are of the same order as that of NGC4486 (Vigo A) and higher. The Hubble constant $H = 75$ km s⁻¹ Mpc⁻¹ and the mean redshifts of clusters of galaxies in distance group 5, given by Abell, were used to determine the radio luminosities.

(f) Most numerous are the clusters of galaxies which do not contain any one dominant galaxy or even 2 or 3 members sharply differing in brightness from the rest. In such clusters the differences in magnitude of bright member galaxies are small (cluster type III according to Bautz and Morgan³). There are 195 such clusters and radio emission is detected at the positions of only 27 of them. Moreover nine radio sources could be located at the positions of clusters purely by chance.

Thus, about 70% of clusters of the first three groups have radio emission associated with them and hence the bright galaxies of these clusters, which are either cD-type, compact, peculiar or close double galaxies, emit at radio wavelengths during at least two-thirds of their lives, or more precisely during the interval of time when they look like galaxies of the named optical types. The duration of the radio emitting phase may be even longer if not all such galaxies pass through this phase.

In clusters of galaxies of groups *d*, *e* and *f*, where the dominating bright member is of type E or there is no outstandingly bright galaxy, the occurrence of radio emission is seven or eight times less frequent than in clusters of the first three groups. Consequently these galaxies emit radio waves for not less than 10% of their lives.

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H₂O, O₃, N₂O and HNO₃ in the arctic stratosphere

MEASUREMENTS of the total amounts and average mixing ratios of a number of stratospheric trace gases at altitudes above 15 km have previously been published¹⁻³. The measurements were derived from submillimetre wavelength emission spectra, recorded on board Concorde 002 during high altitude test flights.

Here we report the preliminary results of further measurements from this aircraft, using an improved experimental system which permitted us to carry out a limb-scanning experiment to deduce not only the total amounts but also the vertical profiles of several gases.

The results were obtained during flights made in May 1973 into the Arctic circle. Prestwick international airport was used as an operations centre, and flights took place during both day and night, reaching a most northerly latitude of 72.5°N. The flight paths were generally triangular, with a rather short northernmost east-west or west-east leg. The present data were obtained on flights on May 16 and 16/17 (night), 1973. Flight altitude was a constant 50,200 feet (15.25 km). Take-off times were 1500 GMT and 2400 GMT. The results reported here were obtained between 65 and 70°N.

The interferograms were recorded once per 8 min, up to an optical path difference equivalent to 0.04 cm⁻¹. These were later Fourier transformed at the National Physical Laboratory (NPL). The atmospheric temperature profiles were obtained from the meteorological sounding network, with kind cooperation from the Meteorological Office. The analysis and spectroscopic assignments of the spectra have been described elsewhere^{1,2,4}. The method used for analysing the concentration profile was a standard limb-scanning method^{5,6}.

Figure 1 shows the initial results obtained, using spectral data from May 16/17, 1973. The diagram shows mixing ratio profiles for H₂O, O₃, HNO₃ and N₂O, a tentative column density for NO₂ and an estimated upper limit for SO₂; it also shows the volume mixing ratios evaluated for each species below 15 km. It is not possible to deduce unambiguously a mixing ratio distribution profile above the flight altitude and so we have quoted only the total

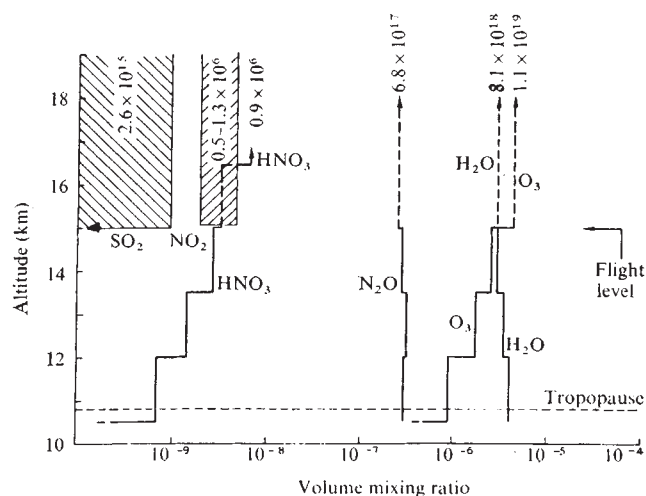


Fig. 1 The vertical distributions of H₂O, O₃, N₂O and HNO₃, at 65–70°N, measured in the limb-scanning experiment on Concorde 002, May 1973, from an altitude of 15 km. Below 15 km, concentrations are quoted explicitly in volume mixing ratio units; above 15 km, total column number densities (cm⁻²) are quoted at the top of each curve. The curve above 15 km represents the (assumed constant) volume mixing ratio equivalent to the quoted column density. At the top left the range of column densities observed for NO₂ is given, and an upper limit to the amount of SO₂.

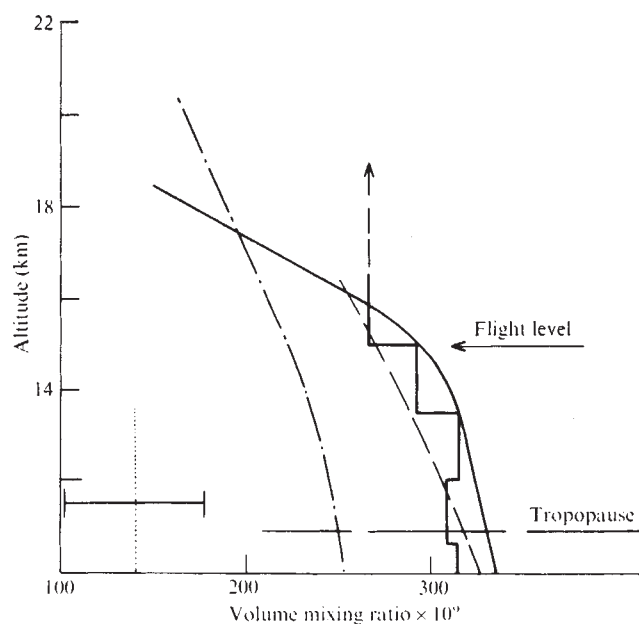


Fig. 2 Results of the inversion procedure for N₂O (May 16/17 at 65–70°N). The stepped curve shows the present results; broken curve, our previous results²; solid curve, some recent results by Murcray *et al.*¹²; bar-dash curve, results by Schutz *et al.*¹³; dotted curve, some earlier results by Murcray *et al.*¹⁴.

column number density above this level. These are the numbers shown at the top of each curve; the values range from 10¹⁹ molecules cm⁻² to less than 10¹⁵ molecules cm⁻². This range of column density may be obtained from a single spectrum, demonstrating the power and dynamic range of radiative methods such as ours. The broken lines with arrows represent the equivalent effective mixing ratios, if constant mixing is assumed, and are shown for comparison.

The shaded areas at the left are for NO₂ and SO₂. In the case of NO₂ we have measured the Q branch at 37.78 cm⁻¹; this, unfortunately, does not yield any information below the horizon, because nearby strong H₂O lines become so intense that the NO₂ Q branch is 'obscured'. Thus information on the column density only has been obtained. The box shown represents the range of values of column number density observed, 0.5 to 1.3 × 10¹⁶ cm⁻² converted to an effective average volume mixing ratio (see above). Some limited evidence is available of a variation between day and night, but more evidence is necessary before it can be regarded as conclusive. In the case of SO₂ there is still uncertainty in our assignments and, moreover, in the value of integrated line strength which should be used. The box thus indicates that we can place an upper limit on the amount of SO₂ above 15 km of 2.6 × 10¹⁵ cm⁻², corresponding to an effective average volume mixing ratio of 1 × 10⁻⁹. This is not an absolute measurement and the true value could be much lower than this.

Below 15 km, we give mixing ratio profiles for H₂O, O₃, HNO₃ and N₂O. The results are given as the average volume mixing ratio in 1.5 km thick layers.

For water vapour, the mixing ratio drops from a value of about 4 × 10⁻⁶–5 × 10⁻⁶ at the tropopause to about 3 × 10⁻⁶ at 15 km and seems to stay near this value above 15 km. We have previously obtained a large amount of information in this region of the stratosphere on the humidity distribution and have invariably found similar behaviour (see Fig. 2 of ref. 1). No diurnal variation was observed.

The ozone profile yields a total amount in good agreement with the limited meteorological network data that were available³ and indicates fairly high mixing ratios in the 11–15 km layer, as might be expected for polar spring maximum

conditions. We are at present analysing similar data taken in October 1973 and hope to compare the spring and autumn profiles. A variation in the amount of ozone between the day and the night flights was observed, which agrees fairly well with meteorological data.

The N_2O profile is basically a constant mixing result, with some indication of a falloff above 15 km. The average mixing ratio in the lower stratosphere is, however, approximately 300×10^{-9} , somewhat higher than the accepted value of 250×10^{-9} . The N_2O results have been replotted in Fig. 2 on a linear scale of mixing ratio and the results of several other workers are shown for comparison. C. B. Farmer and R. A. Toth (private communication) report results similar to Murcray's and our own.

Thus there still seems to be uncertainty about the N_2O profile in the lower stratosphere and it is an open question whether the observed variabilities are a consequence of real atmospheric variations or instrumental effects. It is not possible in the present work to obtain any information about N_2O above about 17–18 km. We did not detect any diurnal effects.

Finally in Fig. 1 we consider the mixing ratio profile obtained for HNO_3 . The mixing ratios seem slightly but significantly higher than those given by Murcray^{8,9} for the same levels but obtained at lower latitudes. Murcray does, however, report higher concentrations in results he has obtained over Alaska at similar latitudes to our own¹⁰ and what is observed may be an effect based on tropopause height and seasonal variations, similar to that found for O_3 . No diurnal variation was detected.

Figure 3 shows the HNO_3 data replotted on a linear mixing ratio scale and compared with other results (the column density above 15 km has been arbitrarily contained below

25 km in order to simulate a layer for the purposes of the diagram. See Fig. 1 for the corresponding column density.) The broken curve is a result by Murcray's group in mid-latitudes⁸, the squares represent the extreme values of a "double-layer" observed by the same group⁹ and the two areas enclosed by polygons circumscribe the range of values obtained, again in mid-latitudes, by Lazarus and coworkers¹¹.

We are at present analysing data for October 1973 and we hope to compare spring and autumn results for HNO_3 in this way.

The picture emerging for HNO_3 seems to be reasonably consistent between the various groups of workers, although at the moment unexplained variations in the total amount do occur, ranging over about a factor of 2. The mean pressure column density seems from our work to be about 2.5×10^{-4} cm atm (in agreement with Murcray *et al.*).

The results reported here are some of the first to be obtained in the Arctic stratosphere, an important part of the atmosphere *vis-à-vis* stratospheric contamination by aircraft because of the typically low tropopause, which means that transpolar and North Atlantic flights spend a large fraction of their time well above the tropopause. They are also the first results from a submillimetre wave limb-scanning experiment.

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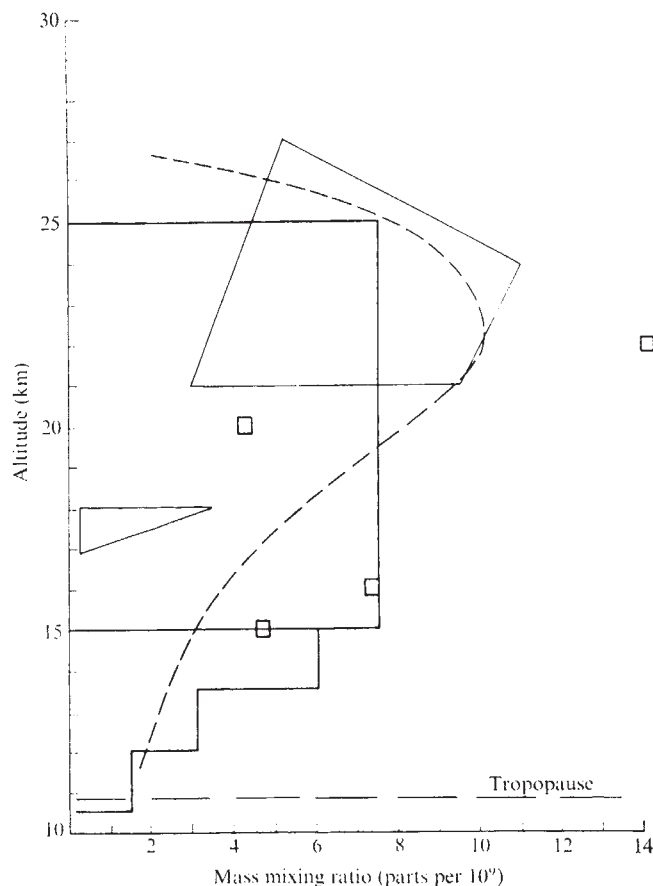


Fig. 3 The results for HNO_3 (May 16/17, 65–70°N) compared with previous measurements. The broken curve is by Murcray *et al.*⁸ as are the squared points⁹; the light triangle and quadrangle enclose points measured by Lazarus *et al.*¹¹. Total column density (10km–top) = 3.14×10^{-4} cm atm.

'Cold spot' in West Africa: anchoring the African plate

VERY low heat flow values of $18 \pm 2 \text{ mW m}^{-2}$ ($0.42 \pm 0.04 \text{ } \mu\text{calorie cm}^{-2} \text{ s}^{-1}$) and $22 \pm 2 \text{ mW m}^{-2}$ ($0.51 \pm 0.05 \text{ } \mu\text{calorie cm}^{-2} \text{ s}^{-1}$) have been determined for two sites 85 km apart on the West African Precambrian shield in the Niger Republic (Table 1).

Temperature surveys were carried out with a thermistor probe in March 1972 in three boreholes, 245 m, 406 m, and 315 m deep, respectively, in western Niger. The thermal

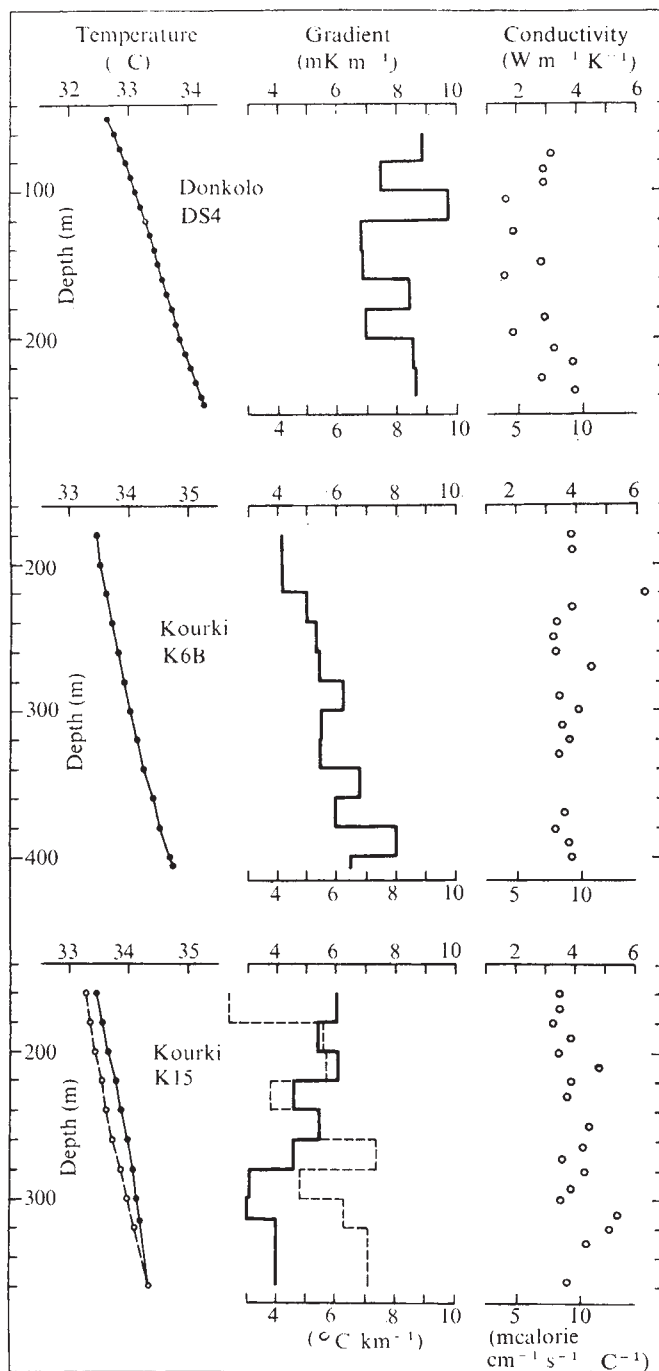


Fig. 1 Temperatures, gradients and conductivities for the analysed portions of the boreholes. In the graphs for K15 the solid curve represents a survey made nine days after drilling had stopped at 315 m, and the dashed line represents equilibrium temperatures predicted from two additional surveys made soon after the hole was deepened to 360 m. The nine-day curve and the predicted equilibrium curve bracket the measured equilibrium profile for K6B which was only 200 m away.

conductivities of solid rock disks, saturated with water before measurement, were subsequently determined on a conventional divided bar apparatus.

Figure 1 shows temperature profiles with depth, temperature gradients calculated for 20-m intervals, and thermal conductivities for the analysed portion of three holes. Two sets of temperature data are shown for site K15. Heat flows were calculated using both K15 gradients; the mean is recorded in Table 1 and agrees very well with the heat flow determined from the nearby site, K6B.

The increasing gradient with depth in K6B (Fig. 1) is puzzling in view of the apparently uniform conductivity of the samples from that hole, and suggests the possibility of recent climatic warming. The consensus of climatological opinion¹, however, is that the region was, if anything, cooling rather than warming during recent times. Furthermore, the Donkolo site does not confirm the speculation of a recent regional warming.

The results of radioactive heat production measurements on aggregate samples of core chips are given in Table 1. The mean of $1.2 \text{ } \mu\text{W m}^{-3}$ for the basement rock at the Kourki site is taken to be representative of the region.

The two sites of the heat flow determinations are both located in Precambrian terrain on the eastern edge of the exposed West African craton. Six K-Ar ages of rock within 100 km of the heat flow sites² range from 2,487–1,206 Myr. The heat flow values reported here substantiate the general observation of low heat flow in Precambrian shields, a result anticipated by Beck and Mustonen³ from temperature measurements in boreholes in Ghana. These west Niger heat flow values are, however, considerably less than the mean heat flows of any of the other shields. The Southern Africa craton provides a useful comparison; there, fifteen measurements range from 36 to 59 mW m^{-2} with an average of 49 mW m^{-2} . The worldwide average of all shields is near 40 mW m^{-2} .

When heat flow data are coupled with information about the distribution of radioactive heat sources, reasonable estimates of temperatures at depth can be made. For a continental shield, Sclater and Francheteau⁴ outline a heat production model based on the petrological concepts of Ringwood⁵ and compatible with observed heat flow and surface heat production data. A surface heat flow in the Sclater-Francheteau model of 40 mW m^{-2} is comprised of 28 mW m^{-2} arising from radioactive heat sources in the outer 400 km of the Earth and 12 mW m^{-2} originating at greater depth.

It is clear that the Niger heat flow could be satisfied entirely by the flux originating above 400 km in the Sclater-Francheteau model, or with appropriate fractions of both the shallow and deeper flux. We reject the former option because it possibly implies a nearly isothermal lower mantle, a condition we think unlikely. Therefore, we have calculated temperature models that include some flux from below 400 km, consistent with two constraints: first, the measured surface heat flow, 20 mW m^{-2} ; and second a near surface radioactive heat source distribution that diminishes exponentially downward from the measured surface value of $1.2 \text{ } \mu\text{W m}^{-3}$. The logarithmic decrement of the near surface heat source function and the temperature dependence of the thermal conductivity remain variable parameters of the models.

Two temperature models for Niger, along with a typical 40 mW m^{-2} shield geotherm and a melting point curve, are shown in Fig. 2. One model, which we have called an 'upper limit' model, represents the likely upper limit for the temperature distribution beneath western Niger, consistent with the listed constraints. It is characterised by a logarithmic decrement of the heat source of $(5 \text{ km})^{-1}$ and a uniform thermal conductivity of $2.5 \text{ W m}^{-1} \text{ K}^{-1}$. We prefer the second model which uses a logarithmic decrement of $(8 \text{ km})^{-1}$ and a modest increase of thermal conductivity

Table 1 Heat flow and heat production data

Site	Latitude	Longitude	Borehole	Depth interval (m)	Heat production ($\mu\text{W m}^{-3}$)	Heat flow (mW m^{-2})
Donkolo	14° 53'N	0° 55'E	DS4	60–245		18 (0.42)
Kourki	14° 25'N	0° 20'E	DS3	140–250	1.8 (4.3)*	
			K6B	180–406		22 (0.52)
			K15	160–358		21 (0.51)
			K6B	60–190	1.3 (3.2)	
			K6B	210–410	1.2 (2.8)	
			K15	50–190	1.1 (2.6)	
			K15	200–360	0.96 (2.3)	
			K16	50–100	1.2 (2.9)	

* Numbers in brackets refer to heat production in 10^{-13} calorie $\text{cm}^{-3} \text{s}^{-1}$ and heat flow in $\mu\text{calorie cm}^{-2} \text{s}^{-1}$.

with temperature, following Schatz and Simmons⁶. Neither of the Niger models reaches the temperatures of the average shield geotherm. Furthermore, unless the entire section of the upper mantle below the West African shield is severely depleted in heat producing isotopes, the heat flow originating below a depth of 400 km must be only 7 mW m^{-2} , about half the corresponding value for the average shield. We therefore must conclude that the Precambrian crust and underlying upper mantle of western Niger probably comprise one of the coldest regions in the outer 400 km of the Earth.

The mechanical consequences of this 'cold spot' are worth brief consideration. The plate tectonic model of Earth dynamics envisions a rigid, mechanically strong lithosphere overlying a weak and deformable asthenosphere. The base of the lithosphere has no rigorous definition; it is commonly equated to the top of the upper mantle seismic low velocity zone. This zone usually begins at depths of 50–150 km, depending on the tectonic setting. We suggest another working definition for the boundary between the lithosphere and the asthenosphere: that depth at which the viscosity of the Earth has diminished from its high surface value to $10^{20} \text{ kg m}^{-1} \text{s}^{-1}$ (10^{21} poise). Such a viscosity for the asthenosphere is suggested by postglacial rebound, and also

by the velocities of lithospheric plate motion over the asthenosphere.

We have calculated the viscosity as a function of temperature, and thus of depth, for the temperature models shown in Fig. 2. We have followed the development of Weertman⁷, which relates the viscosity to temperature-dependent creep and dislocation glide. The calculations assume a stress of 10^4 N m^{-2} (0.1 bar) and the melting curve shown in Fig. 2. The calculated viscosity profiles also are shown in Fig. 2. The model for the average shield temperature implies a lithospheric thickness of some 175 km for a typical shield, a value quite consistent with seismically determined values reported for various shields. The two models for West Africa suggest that the lithosphere there extends to depths well below 400 km; whether an asthenosphere is developed at greater depths is somewhat uncertain. Our conclusion is that the lithosphere is very thick, and that the asthenosphere is very thin or absent beneath West Africa.

The logical consequence of a thick lithosphere and a poorly developed or absent asthenosphere would be that the motion of the lithosphere would be impeded. In the extreme, the plate would be rendered immobile. Burke and Wilson⁸ have indeed suggested that such has been the case for the African plate since the early Miocene.

Has the African plate run aground?

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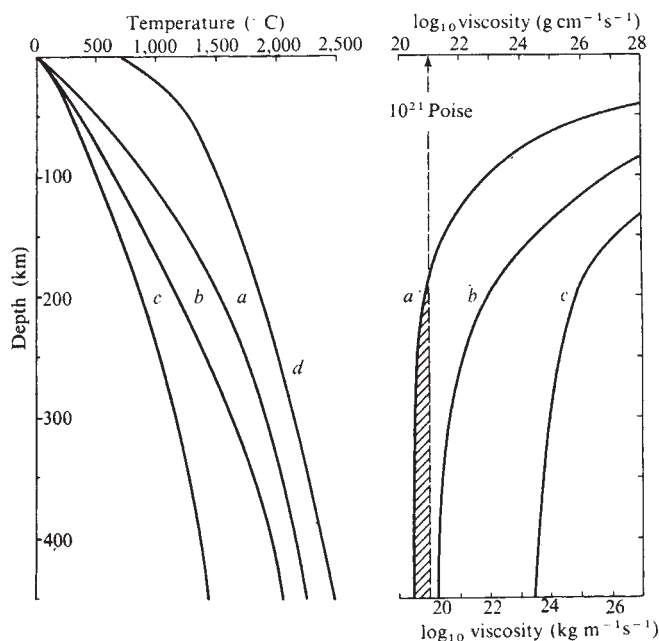


Fig. 2 The variation of temperature and viscosity with depth for three heat flow-heat production models: a, Average shield; b, Niger upper limit; c, the preferred Niger model, d, melting point.

Magnetic behaviour of some partially unmixed titanomagnetites

THE remanent magnetisation of igneous rocks is mainly carried by titanomagnetites. To obtain reliable information about the Earth's field during the past it is necessary to understand the mechanisms involved in the acquisition of remanence and in any subsequent changes. Much information has been obtained by studying basalts, but this approach is limited because of unknown factors such as grain size, degree of subsequent oxidation and impurities present. Synthetic, sintered samples of known composition have been used by many workers in order to avoid these difficulties.

In our present work, sintered titanomagnetite, $\text{Fe}_{3-x}\text{Ti}_x\text{O}_4$ (with $x=0.5$, denoted TM50) was used to prepare simulated average basalt by dispersing a known quantity of a selected particle size into a silicate nonmagnetic matrix (powdered Pyrex or silica). This study was undertaken in order to investigate reversals of magnetisation observed in natural basalts¹. The simulated sample showed a partial reversal of remanence when cooled in zero field. The study was then extended to the compositions TM40 ($x=0.4$), and TM60 ($x=0.6$).

The samples were prepared by a double sintering procedure² at $1,350^\circ\text{C}$ in a sealed mullite tube filled with a mixture of CO_2 -CO which acted as an oxygen buffer and at the same time aided the oxygen transfer between Fe and Fe_2O_3 in the initial mixture consisting of Fe, Fe_2O_3 , and TiO_2 . All samples were cooled at approximately the same rate given by the normal cooling rate of a massive furnace. It took about 15 h for the furnace to cool from $1,350^\circ\text{C}$ to room temperature. The samples were chemically analysed (% of Fe^{2+} by wet chemical analyses, Fe:Ti ratio by electronprobe microanalyses) and their phase uniformity checked by X-ray analyses using Debye-Scherrer and Guinier-de Wolff cameras. All samples were nearly stoichiometric and free of stray phases. Initial susceptibility, and saturation magnetisation-temperature curve confirmed this. The size of individual crystallites (grains) in the densely sintered tablets ranged from about 20 to $160\text{ }\mu\text{m}$, grains of

$40\text{ }\mu\text{m}$ being most abundant.

A part of the TM50 material was powdered and fractionated into various particle sizes by means of a centrifugal dust classifier. But, in this study only the fraction containing particles from 20 to about $50\text{ }\mu\text{m}$ was used. It was mixed with specially dried powdered Pyrex to give 3.5% by volume of the titanomagnetite in the non-magnetic matrix. The mixture was enclosed in a Pyrex capsule. This was evacuated and outgassed and the capsule sealed off in such a way that the magnetic particles were immobilised.

The sample was given an isothermal remanent magnetisation (IRM) at room temperature in a direct field of 5 kOe. The result of continuous thermal demagnetisation in zero field obtained by means of an astatic magnetometer

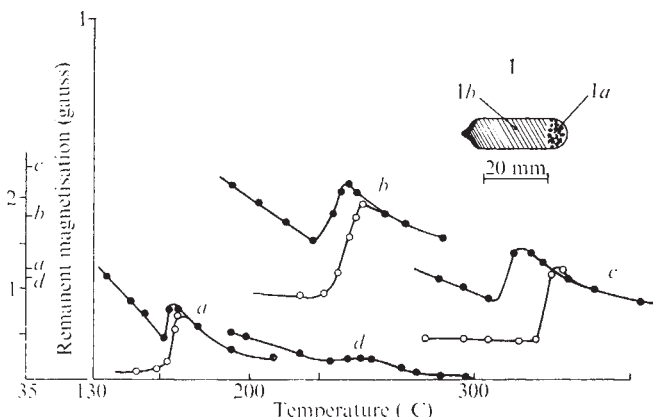


Fig. 2 Magnetic remanence as a function of temperature for *a*, TM60, *b*, TM50, and *c*, TM40. *d*, TM50 annealed at $1,100^\circ\text{C}$. Heating curve (●), cooling curve (○). The values of remanence at 35°C are shown on the left vertical axis (reduced four times). The Curie temperatures determined from susceptibility measurements are indicated on the temperature axis as bold marks. Inset 1: Pyrex (quartz) capsule with TM40, 50, and 60; 1*a*, coarse particles of titanomagnetite; 1*b*, powdered Pyrex (quartz).

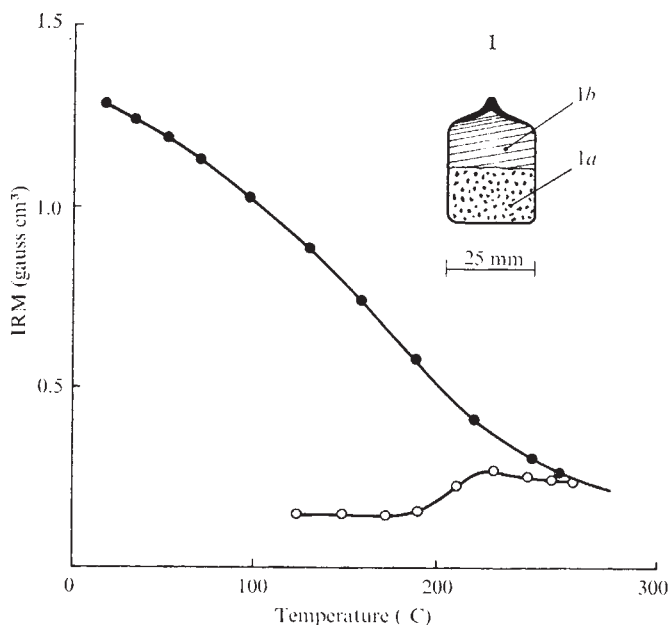


Fig. 1 Magnetic remanence as a function of temperature for simulated basalt. Heating curve (●), cooling curve (○). Inset 1: Pyrex capsule with the simulated basalt; 1*a*, layer of TM50 particles dispersed in powdered Pyrex; 1*b*, protective layer of powdered Pyrex.

next to the heating furnace³ is shown in Fig. 1. The two Curie temperatures show that while the bulk of the material is TM50, magnetite is also present. The amount of magnetite proved to be under the detection range of X-ray analyses, while low field susceptibility and saturation magnetisation-temperature curves suggest a very small quantity of magnetite (possibly under 1% by weight). If the demagnetisation was stopped at about 280°C (after the Curie temperature of TM50 was reached) and the sample was then cooled in zero field the remanent magnetisation showed a partial reversal on cooling through the Curie point. The same result was observed after prolonged heating to higher temperatures.

To investigate further the partial reversal, samples of TM40, TM50 and TM60 were prepared in a time-saving way as indicated in the inset to Fig. 2. All the magnetic sample in the form of relatively large pieces broken off from the original tablet (ranging in size from about 0.1 to 1.0 mm) was confined to the bottom space of a Pyrex (or quartz) ampoule. The three samples were then given an IRM in a field of 5 kOe. The results of continuous thermal demagnetisation are shown in Fig. 2. The curves exhibit a sudden increase of remanence at the Curie temperature of each composition respectively and the final Curie temperature of magnetite. If the samples were cooled after the first Curie point had been reached and before total demagnetisation occurred, a very sharp decrease in remanence was observed at a slightly higher temperature than the 'jump' observed on heating. The coarsely crushed sample of TM50 was then annealed at $1,100^\circ\text{C}$ for 3 h in an attempt to resorb the small quantity of magnetite. To avoid any appre-

ciable unmixing occurring on cooling, the sample was rapidly removed from the hot furnace into a water-cooled brass jacket. This was successful, as in the repeat demagnetisation experiment the strange behaviour in the vicinity of the Curie temperature of TM50 practically disappeared (Fig. 2 curve *d*). It is noteworthy that the initial value of IRM exhibited by the sample at room temperature (see point *d* on the left vertical axis in Fig. 2) was very much smaller than previously. All these results suggest the possibility of a strong coupling between the magnetite regions present and the original titanomagnetite matrix.

The partial reversal observed on cooling of our simulated basalt seems to be similar to partial reversals demonstrated in natural basalts^{1,4-6} and in synthetic TM60 (ref. 7). This effect has been explained by negative magnetostatic coupling between the original spinel and a partially-oxidised cation-deficient spinel with higher T_c . In our samples, however, oxidation was ruled out. So, this explanation cannot account for the features of the curves shown in Fig. 2. We believe that all our observations are related to the same phenomenon—the presence of segregated magnetite and the resulting coupling. Small regions of magnetite are most likely to be the product of subsolvus segregation (in petrological terminology usually called ‘unmixing’) from the originally homogeneous single phase titanomagnetite. The curve confining the miscibility gap in the magnetite–ulvöspinel solid solution series is reported to be of the simple ‘hoop-shaped’ type⁸. It is known, however, that the ‘two spinels’ field in the appropriate phase diagram⁸ is still rather tentatively marked and much less is known about the kinetics of unmixing. Nevertheless, the phase diagram shows that the critical temperature for unmixing for the three compositions studied here should lie between 500° and 700° C. Unmixing in our samples is therefore likely to have taken place during the relatively slow cooling after their final sintering.

The correlation between the position of the jump and the Curie temperatures is obvious. The temperatures marking the onset of the jump on the heating curves correspond approximately to 160°, 230°, and 315° C for TM60, 50, and 40 respectively. The corresponding Curie temperatures determined for the latter samples from the susceptibility-temperature curves were 159°, 227° and 320° C. The reproducibility of the curves in Fig. 2 shows that no unmixing is taking place during the thermal demagnetisation. The jump is also reversible on cooling except for a hysteresis which grows with increasing x value.

The magnetic behaviour of the samples in our experiments can be phenomenologically explained in the following way. Below the Curie point of the matrix its permeability is high and some of the magnetic lines of force emanating from the magnetite regions are trapped in the matrix of the parent titanomagnetite. This results in a reduced amount being sensed by the astatic magnetometer. On passing the Curie point the permeability of the matrix drops, so that the lines of force emanating from the magnetite inclusions spring out of the sample and the magnetometer records a sudden increase in moment. When the sample is cooled the permeability rises sharply at the Curie point of the matrix and the lines of force from the inclusions are once more partially trapped and the decrease in moment is produced. The onset of these downward jumps which occurs at slightly higher temperatures may indicate that the regions adjacent to the magnetite inclusions could be slightly richer in iron than the bulk of the matrix. Some other mechanism might account for these hystereses, however, such as temperature-dependent reordering of cations. Research into this possibility is under way. In fact, we have observed similar hystereses on the susceptibility-temperature curves.

The simulated rock does not show the relaxation on heating through the Curie point. This may be associated with the different history of this sample, in that the titan-

magnetite particles are smaller, mutually separated by non-magnetic material and strained because of grinding. More experimental data are needed.

It seems that measurements of magnetic remanence provide a very sensitive method of studying the process of unmixing in titanomagnetites from the very initial stage. It may also prove valuable for studying the kinetics of unmixing. We believe that further research carried along these lines will have petrological as well as palaeomagnetic significance as the process of unmixing is undoubtedly very common in natural basalts.

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Humic substances from seawater

SINCE the first spectroscopic observation of humic substances in seawater¹, it has been presumed that they constitute a considerable fraction of the dissolved organic matter in the sea²⁻⁴. Base-soluble organics were first isolated from seawater in 1958 (ref. 5), but the chemical composition and source of these materials has remained speculative and controversial^{3,6-9}. We here present evidence that humic substances in the Sargasso Sea display bulk characteristics which differ from those of humics from coastal waters and especially from those of humic substances isolated from soils and marine sediments.

Water from the north-western Sargasso Sea (surface and 1,500 m), and coastal water off Woods Hole, Massachusetts, has been analysed. Batches of seawater (400 l each) were acidified (pH 2, HCl) and passed through Amberlite XAD-2 resin¹⁰. (The resin was cleaned prior to use by batch extraction in refluxing benzene until NH₄OH, ethanol, and methylene chloride concentrated eluents showed no ultraviolet absorption. An extraction efficiency of 95.2% was determined when isolated humic substances were redissolved and adsorbed on Amberlite XAD-2 from salt water (pH 2) at 1.7 bed volumes per minute.) Salt was eluted with distilled water and humic materials were eluted with NH₄OH (pH 11.6) and recovered by freeze-drying. The humic materials were further fractionated by dissolving the fulvic acid (FA) in 0.01N HCl and the humic acid (HA) in NH₄OH. Freeze-drying these fractions yielded FA:HA ratios of 97:3 (by weight) in Sargasso surface water, 78:22 in

Sargasso deep water, and 85:15 in coastal water. This indicates variations in the composition of humic substances in different water regimes.

The elemental composition of FA from Sargasso surface water is 49.98% C, 6.76% H, 6.40% N, 0.46% S and 36.40% O, by difference (corrected for ash, 3.4%). The C:H ratio of 7.4 is within the range of terrestrial¹¹ and marine sedimentary⁹ FA, but the oxygen content is exceptionally low. The sulfur content is lower than that of marine sedimentary FA (ref. 9).

The ultraviolet absorption of FA from Sargasso surface and coastal water increases with decreasing wavelength, without prominent features. In general, the absorbance of soil FA (ref. 11) is about five times that of FA from coastal water and about an order of magnitude greater than the absorbance of FA from the Sargasso Sea. The ultraviolet-visual absorption ratio for Sargasso FA is higher than for coastal water FA and is much greater than reported values for soil¹¹ and sedimentary⁹ FA. This suggests that there are fewer highly conjugated chromophores in FA which is dissolved in marine waters, especially in the Sargasso Sea.

The NMR spectrum of Sargasso FA (D₂O with methanol internal standard) shows a predominance of paraffinic methylene protons (1.0 to 1.3 parts per million—p.p.m.) and protons adjacent to functional groups (2.1 to 2.8 p.p.m.), with a minor fraction of aromatic protons (7.6 to 7.9 p.p.m.). Integration gives relative areas of 15:10:1, respectively.

The $\delta^{13}\text{C}$ values for Sargasso surface water FA, coastal water FA and coastal water HA are -22.79% , -23.72% and -22.78% , respectively. ($\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C})_{\text{sample}}/({}^{13}\text{C}/{}^{12}\text{C})_{\text{standard}}] - 1 \times 1,000$. Standard is Chicago Pee Dee Belemnite.) These values are within the range of $\delta^{13}\text{C}$ values for marine sedimentary FA (ref. 9) and are less negative than those of soil FA (-25% to -27%). This supports the idea of a marine source for the dissolved humic substances.

An equivalent weight of 473 g/equivalent was calculated for Sargasso surface FA after titration of a NaOH solution of this material with acid. There was an absence of inflections on the main titration curve, which indicates the presence of functional groups displaying a wide range of pK_a values.

The molecular weight (MW) distributions of Sargasso and coastal water FA (Fig. 1) were determined by elution from

Sephadex G-10, G-15, and G-25 gels with a 0.05M NH_4HCO_3 buffer (pH 7.2). The bulk of the material is in the lowest MW fraction (< 700), especially for the Sargasso FA. This is unusual when compared to the higher molecular weights of soil FA (ref. 12) and marine sedimentary FA (refs 7 and 13). Complete elution of samples within one column volume, and elution of rechromatographed fractions in their same fraction volumes demonstrate that size fractionation is not seriously hindered by gel-solute interactions.

These initial studies show that FA isolated from sea water by this method, differs from soil and marine sedimentary FA, and indicate a marine origin for the dissolved humic substances. Further work is in progress on the Sargasso FA mixture.

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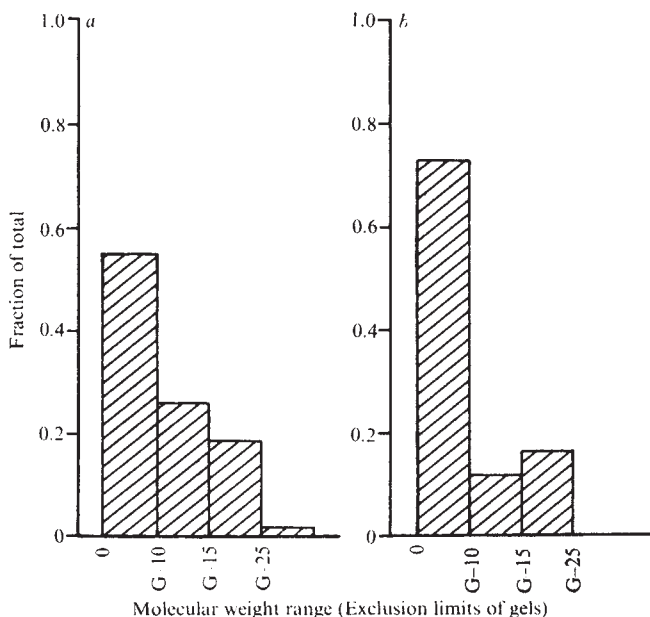


Fig. 1 Molecular weight distributions of fulvic acid from the surface water of the Sargasso Sea, and coastal water fulvic acid, with molecular weight ranges (given in exclusion limits of gels). Sephadex G-10, G-15 and G-25 gel exclusion limits are approximately 700, 1,500 and 5,000, respectively. a, Coastal water; b, Sargasso Sea.

27-day cycle in the rainfall at Los Angeles

IN each of two Los Angeles rain seasons (Table 1) rainfall was concentrated in the first half of arbitrarily chosen 27-d intervals. This suggests the existence of a rainfall component with a period near 27 d. The phase was roughly constant throughout the 5-yr interval from mid-1966 to mid-1971. Usually, however, over the preceding 46 yr studied, the phase changed from year to year, only occasionally remaining constant for 2 or 3 yr. Figure 1 is a

Table 1 Daily rainfall at downtown Los Angeles weather station tabulated for nine successive intervals (A-I) of 27 d starting October 5, 1966 (left side) and August 25, 1967 (right side).

123456789012345678901234567	123456789012345678901234567
8	1
33 42	2
	836
8 4 1	30
	1 0
12	0
2	34
20 2 11	0
	1

At top of table last digit of each of the 27 d in cycle is given in column corresponding to that cycle day. In table integer 0 is 0.15–0.25 in., 1 is 0.25–0.50, 2 is 0.5–1.0, . . . 8 is 3.5–4.0, and 9 is ≥ 4.0 . A blank indicates < 0.15 inches.

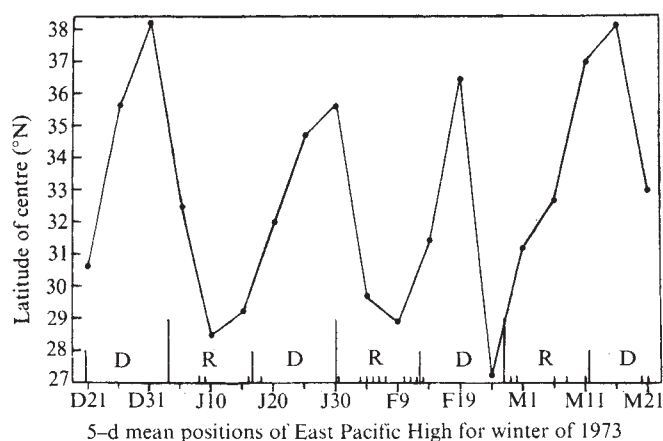


Fig. 1 Latitude time plot of 5-d mean position of surface East Pacific high pressure cell for winter of 1973. D, J, F, and M are abbreviations for the months (December through March). The symbol R denotes rainier halves and D drier halves of successive 27-d intervals. The upper tick marks indicate days it actually rained in Los Angeles.

graph of the 5-d mean positions, plotted on the third day, of the principal centre, or cell of highest pressure of the semipermanent East Pacific high pressure cell in the region 19 to 50°N and 120 to 165°W for the 1973 winter (Los Angeles is at 34°3'N and 118°14'W). On average, the cell was more southward during rainier halves and more northward during drier halves of the 27-d intervals. The oscillation seems to be greater and more regular in winter than in other seasons.

This rainfall cycle might be related to a cyclic latitudinal oscillation of the jet stream at west coast longitudes. This oscillation might, in turn, cause a cyclic latitudinal oscillation of the resident East Pacific high pressure cell. The oscillation might then modulate the rainfall. The oscillation's phase would then determine whether Pacific storms move inland and eastward across Northern California or whether the storms move southwards. The respective positions about which the jet stream and Pacific high cell oscillate move farther south¹ as the rain season progresses with greater effects on conditions in Southern California (population 12×10^6) as the season progresses.

Records of daily precipitation for 1921 to 1971 for Los Angeles, Santa Barbara (34°25'N, 119°42'W) and San Diego (32°43'N, 117°9'W) were obtained from the National Climatic Center, Asheville, North Carolina. Additional Los Angeles Civic Center data from 1900 to 1920 were obtained from the Los Angeles office of the National Weather Service. The mean annual rainfall for Santa Barbara is 17.63 inches compared with 14.42 inches for Los Angeles (Table 2) and 9.76 inches for San Diego. The lower frequency part of the power spectrum for each of these cities is plotted in Fig. 2. The time series used for each spectrum was the cube root of the daily rainfall²⁻⁴. The entire continuous record was used to make each power spectrum and the data were also detrended. The Blackman-Tukey

method⁵ and the Tukey spectral window were used to compute the power spectrum, with 250 lags (equal maximum lag number). Twice as many spectral estimates as lags were computed⁶. The spectral estimates are statistically correlated in a band but greater resolution is obtained.

The 80% confidence interval is used as the standard of significance⁵. For the five curves the heights of the peaks at 54 d and 27 d (and for the 13.5-d peak in most cases) are equal to or larger than the 80% confidence interval and are therefore considered to be significant. There is a significant peak at almost exactly 27.0 d. The frequency for that spectral estimate is 0.037 cycles d⁻¹ which gives

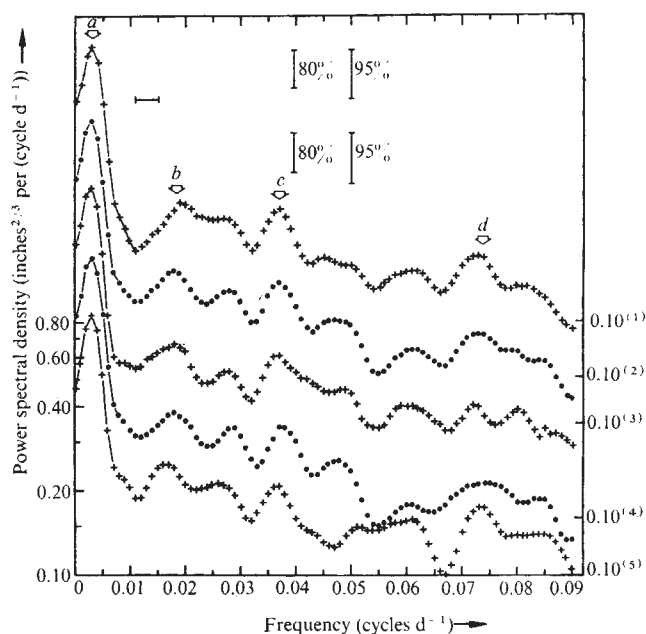


Fig. 2 Power spectra of Southern California rainfall records. The five superimposed curves, numbered 1 to 5 from top to bottom, give lower frequency part of power spectral density as a function of frequency. The left-side ordinate scale of 0.10 to 0.80 is for curve 5. The 0.10 level for each curve is shown on the right side. Curves 1, 2, and 3 represent, respectively, spectra of Santa Barbara, Los Angeles, and San Diego, for the interval December 1927 through 1971 and each have 129 degrees of freedom. Curves 4 and 5 are spectra for Los Angeles for years 1936-1971 and 1900-1935, respectively, and each have 105 degrees of freedom. The vertical bars at very top are 80% and 95% confidence intervals for curves 1, 2, and 3. Below them are confidence intervals for curves 4 and 5. The horizontal bar in upper left corner is spectral bandwidth. Arrows a, b, c and d, denote peaks at 365, 54, 27 and 13.5 d, respectively.

$1/0.037 = 27.03$ d. The spectra for Los Angeles in curves 4 and 5 were obtained for time intervals completely independent from each other. San Diego is 180 miles to the south-east of Santa Barbara. The 27-d and 54-d peaks at Santa Barbara meet the very stringent 95% confidence interval standard⁶. Santa Barbara is 80 miles west and 30 miles north of downtown Los Angeles and is closer to the central longitude of the semi-permanent, East Pacific high cell. Thus, the storm blocking capability of this cell may be more effective at Santa Barbara, producing relatively larger 27- and 54-d peaks there. This possibility suggests that the closer the cell's average longitudinal position is to the west coast, the more dominant is the 27-d rainfall cycle.

The curves suggest two sets of harmonically related bands. The harmonics of "Set A" are separated by 0.009 cycles d⁻¹ (fundamental period ~ 108 d). The harmonics of "Set B" are separated by 0.012 cycles d⁻¹ (fundamental 81 d). In

Table 2 Monthly rainfall, in inches, at downtown Los Angeles station, averaged over the years 1921 through 1971.

January	2.84	July	0.00
February	2.93	August	0.03
March	2.11	September	0.25
April	1.34	October	0.38
May	0.22	November	1.56
June	0.07	December	2.69

neithercase is the fundamental marked by a peak. The first apparent peak in Set A is at 54 d and in Set B it is at 27 d. The peaks at 27 d and 13.5 d are effectively common to both sets. There are no peaks at Set A harmonics at 0.056 cycles d^{-1} and 0.065 cycles d^{-1} . Curves 1, 2, and 3 contain a mixing of both sets. The Los Angeles spectrum for 1936–1971, curve 4, is dominated by Set A and that for 1900–1935, curve 5, contains more of Set B than curve 4. On curve 5 there are indications of contributions but no peak by the Set B fundamental at 0.012 cycles d^{-1} and by its first harmonic at 0.024 cycles d^{-1} . These contributions partially fill in regions that are troughs at those same locations on curve 4 and seem to shift the two nearby peaks to lower frequencies on curve 5.

The Los Angeles computer tape was used in simulating forecasts for the past 50 yr (Table 3). The oscillation of the Pacific high cell (Fig. 1) suggests the primacy of the 27-d cycle. The other two large significant peaks, at 54 d and 13.5 d, are related to the 27-d cycle by factors of 2. The forecast was simulated by finding the early trend in the

Table 3 Results of simulated forecasts using 27-d cycles for each of 50 Los Angeles rain seasons ending in approximately mid-summer of year shown in column A

A	B	C	D	E
1922	1.57	0.96	1.03	306
1923	0.94	0.89	0.83	348
1924	1.76	0.77	0.68	35
1925	0.73	0.90	0.79	349
1926	2.42	0.93	1.09	337
1927	1.73	0.93	1.09	337
1928	0.47	0.92	0.97	362
1929	0.34	0.87	0.83	347
1930	0.0 N	0.0	0.0	0
1931	9.90	0.94	0.97	16
1932	1.12	0.97	0.99	355
1933	3.73	0.86	0.79	40
1934	0.01	0.70	0.49	62
1935	3.12	1.01	0.98	327
1936	0.16	0.70	0.49	62
1937	1.31	0.86	0.88	2
1938	5.68	0.86	0.88	42
1939	2.11	0.98	1.08	46
1940	0.07	0.77	0.64	54
1941	1.27	1.00	1.06	332
1942	1.22	0.86	0.81	346
1943	1.16	0.89	1.00	364
1944	6.64	0.85	0.81	8
1945	1.49	0.84	0.79	344
1946	0.47	0.88	0.97	365
1947	1.13	0.91	1.01	308
1948	0.0 N	0.0	0.0	0
1949	1.96	0.95	0.95	21
1950	0.91	0.90	0.79	349
1951	2.29	0.85	0.68	65
1952	1.67	1.00	1.06	332
1953	0.13	0.92	0.97	362
1954	0.97	0.77	0.70	53
1955	1.45	0.91	0.77	350
1956	9.90	0.84	0.77	4
1957	3.40	0.84	0.75	37
1958	1.40	0.87	0.83	347
1959	0.0 N	0.0	0.0	0
1960	7.52	0.98	1.08	46
1961	9.90	0.87	0.87	341
1962	2.29	0.98	1.03	20
1963	0.0 N	0.0	0.0	0
1964	0.44	1.01	0.91	326
1965	0.59	1.01	0.98	327
1966	0.27	0.97	0.99	355
1967	2.11	0.86	0.81	346
1968	3.50	0.98	1.04	333
1969	2.54	0.73	0.57	32
1970	0.10	0.86	1.03	71
1971	3.95	0.88	0.97	365

Column B gives rainier-to-drier half ratio for predicted intervals. Column C gives same ratio using climatology statistics consisting of total number of days of rainfall on each calendar date for 1919–1973. Column D gives the ratio using statistics of average amount of rainfall on each calendar date from 1919–1973. Column E gives day of year (1 to 365 or 366) on which each forecast began, 0 in column E indicates no forecast made.

27 cycle days (using two 27-d periods) that revealed the pattern that might continue. The first day after September 23 with ≥ 0.26 inches of rain or ≥ 0.26 inches total in two adjacent days was taken as point 1. The area in the next 27-d period under point 1 (23 to 30 d later) was searched for nearby days on at least one of which there was ≥ 0.16 inches. If this condition was not met, then a second point was searched for. When the day with a repeating pattern was found, it was taken as the "centre" day of the 13.5 days in a rainier half cycle. Only rain in this half cycle in cycles (to June 30) after the ones used in the trend establishment was totalled, and the ratio to rain in the other half cycle was calculated and printed in column B. The summation of the rains for the ratio in column B was started on the first day of the first drier half in the cycles to be included. This day was the eighth day after the centre day of the second 27-d interval used in establishing the early trend. If there was no more than 0.25 inches on any day between September 24 and December 31, then no forecast was made (Next to column B and 0.0 in column B). In a real-time forecast in the early autumn, one must distinguish and eliminate the very infrequent local thunderstorms and Sonoras (storms from Baja, Mexico). The average for the ratio in column B is 2.40 and 31 yr, or 67.4%, exhibited a ratio of 1.10 or greater, the criterion used for "success". Most forecasts (58.7%) were started (column E) before January 1.

Climatological rainfall statistics for Los Angeles, based on 1919–1973, were available at UCLA. We have examined climatological daily averages and determined the positive skill score over climatology from the formula^{7,8} $(C-S)/(T-S)$, where C is the number of correct forecasts (ratio in column B ≥ 1.10), and T is the total number of forecasts made. S is the number of those correct forecasts in column B that were also correct when the climatology statistics were used in column C or D in place of actual rainfall. If we take⁷ 1.05 or above, instead of 1.10, as an indication of success in columns C and D, then $S=6$ for column D and S is still zero for column C. For column C (using the number of rainfall days) the positive skill over climatology is 0.67 and for column D (using rainfall calendar date averages), it is then 0.63. The average of the two comparisons is 0.65. Using the same method as above, real-time forecasts were made for the 1972–73 and 1973–74 Los Angeles rain seasons and gave, respectively, 3.01 and 4.76 times as much rain for the predicted rainier halves as for the predicted drier halves of the 27-d cycles. The 1973–74 forecast was monitored by the Los Angeles office of the National Weather Service.

Rainfall may occasionally be scant within an entire 27-d interval. This may occur if the 54-d cyclic component is then more effective or if storms are blocked by a strong ridge of high pressure which sometimes forms over the western states and persists for a few weeks. When the southern part of the landward extension of the East Pacific high is over Los Angeles, as the cell moves northward during the drier half, the wind direction is from the east since the high circulates in a clockwise sense. Then the East Pacific high over Los Angeles can help create the Santa Ana wind condition (foehns) and higher temperatures from compressional effects as air flows downhill toward the coast. Rain washes pollutants out of the atmosphere and cold fronts associated with storms do not favour formation of low-temperature inversion layers. For the 1972–73 rain season figures obtained from the Los Angeles County Air Pollution Control District show that, on average, the ozone level was 43.5% higher and the nitrogen oxides 11.3% higher during the predicted drier halves of the five principal 27-d rainfall cycles.

Our analysis indicates that the phase of the 27-d cycle usually remains the same for most or all of a particular rain season, and that the phase changes usually occur be-

tween rain seasons. Some very small effects on rainfall are associated with both the full moon and new moon which occur every 14.7 d (refs 4, 9). This particular lunar phase effect would give roughly equal rainfall contributions (14.7 d apart) to the drier and rainier halves of the Los Angeles 27-d rain cycle and is probably not related to it. The Sun and solar sector structure also produce atmospheric effects¹⁰. But our studies of the rainfall cycle's phase indicate that it is not simply driven by either solar or lunar processes. If the periodicity is of solar or lunar origin, then its phase must be determined by another mechanism.

Just off the northwest coast of the United States, confluence (narrow, strong jet stream) is associated with the Gulf of Alaska surface low surmounting the eastern cell of the Pacific high¹¹. These unusual circumstances provide very good conditions for high zonal index so storm tracks can stay north of Southern California a long time. We now consider the possibility that the spectral peaks in Fig. 2 arise from resonances of the atmospheric subsystem regulating the weather, including the Gulf low and the Pacific high, in the Eastern Pacific region at northern hemispheric mid-latitudes. The spectra suggest the possibility that this resonance is excited by time dependent processes with energy at frequencies closer to higher harmonics than to the fundamental of the subsystem and closer to some harmonics than to others. Since atmospheric systems are "deformable", we might expect a somewhat different fundamental and a different set of harmonics in years when the atmospheric conditions were different. The differences in the spectra in curves 4 (1936-1971) and 5 (1900-1935) may be the result of a long term change in the state of the atmosphere. Thus, Conover¹², citing work by Petterssen¹³, states that the global trend of rising temperatures diminished after about 1937-1940. He relates changes in temperature trends to changes in the mean atmospheric state of meridional flow relative to zonal circulation.

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Concentrating solutes with membranes containing carriers

WE have developed membranes which can rapidly concentrate a specific solute against its concentration gradient. These membranes depend on mobile carriers and are examples of *in vitro* systems which exhibit characteristics of biological transport. When combined with the 'liquid surfactant membrane' geometry, they offer a method for wide classes of large scale separations. We show here how our specific results provide a blueprint for designing additional membrane systems.

The membranes operating in our laboratories at present are summarised in Table 1. These membranes depend on mobile carriers which react rapidly and selectively with the solutes being transported. Two mechanisms are involved. In the first (Fig. 1), the flux of the protons which supply the energy is in the opposite direction to the flux of the solute being moved against its gradient, resulting in counter-transport^{1,2}. The second mechanism is analogous to that shown in Fig. 1, except that both the protons and the solute being separated combine with the carrier on the same side of the membrane. As a result, the two fluxes are in the same direction, resulting in co-transport. Moreover, because all of these membranes are chemically well defined, they can be understood on a molecular basis, and the diffusion and chemical reaction responsible for their operation can therefore be studied in detail³.

Additional membrane systems can be developed in a straightforward manner. An organic solution containing a complexing agent for the relevant solute must be chosen. This solution and the complexing agent will become the membrane material and the mobile carrier, respectively. The degree of complex formation must vary strongly with pH, and both carrier and complex must be soluble in the membrane solution but insoluble in aqueous acid and base. One then runs two extractions sequentially. For example, a solution of 500 parts per million (p.p.m.) mercuric ion in 2 M NaCl and 1 M HCl is mixed with a membrane solution of 10% trioctylamine in *o*-xylene. The mercuric ion and the protons complex with the amine and dissolve in the organic phase. When this organic phase is

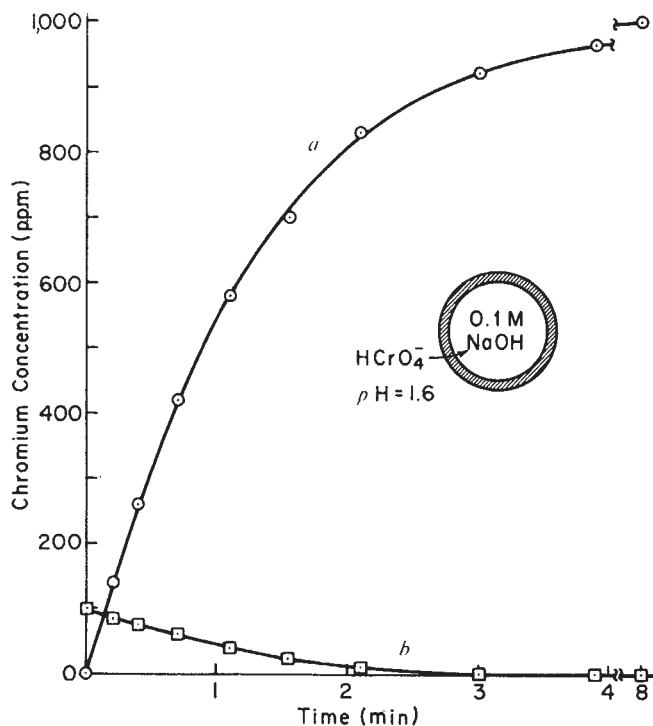


Fig. 1 Counter-transport mechanism involving a mobile carrier.

Table 1 Existing membrane systems*

Solute	Carrier	Transport mechanism	Energy	Remarks
Na ⁺ , K ⁺ , Li ⁺ , Cs ⁺	Monensin in octanol	counter-	HCl into NaOH	Selective for Na ⁺ ; strong biological parallel
Na ⁺ , K ⁺ , Li ⁺ , Cs ⁺	Cholanic acid in octanol	counter-	HCl into NaOH	Non-selective analogue of above
Cu ²⁺	$\begin{array}{c} \text{O} \quad \text{O} \\ \quad \\ \phi\text{CCH}_2\text{CCH}_3 \end{array}$ in chloroform or in carbon tetrachloride	counter-	HCl into NH ₄ OH	Works best at 0.01 M Cu ²⁺ ; selective for Cu ²⁺ over Ni ²⁺ and Co ²⁺ ; carriers poisoned by Fe ³⁺
	$\begin{array}{c} \text{O} \quad \text{O} \\ \quad \\ \phi\text{CCH}_2\text{CCF}_3 \end{array}$ in xylene	counter-	HCl into NaOH	
Zn ²⁺ Pb ²⁺ Hg ²⁺	$\begin{array}{c} \text{O} \quad \text{O} \\ \quad \\ \phi\text{CCH}_2\text{C}\phi \end{array}$ in chloroform	counter-	0.5 N HCl into pH 8.5 citrate	Works best at high dilution
	Dithizone in carbon tetrachloride		HCl into NaOH	Selectivity depends on pH difference
	Trioctylamine in xylene	co-	HCl into NaOH	Works best at 1,000 p.p.m. with 2 M Na ⁺ on both sides
Cl ⁻	Trioctylamine in xylene	co-	HCl into NaOH	Selectivity depends on pH difference
SO ₄ ²⁻			H ₂ SO ₄ into NaOH	
Cr ₂ O ₇ ²⁻	Trioctylamine in xylene	co-	H ₂ Cr ₂ O ₇ into NaOH	Potential for treatment of plating wastes
	Tridodecylamine in hydrocarbon oils	co-	H ₂ SO ₄ into NaOH	

* All these systems have been checked by double extraction and in a membrane geometry.

extracted with 500 p.p.m. mercuric ion in 2 M NaCl and 0.3 M NaOH, the mercury concentration of the basic solution is increased. The two extractions produce a net transfer of

material, and therefore this chemical system provides a potential separation process. As a thin layer dividing two aqueous solutions, it will function as a membrane capable of concentrating a specific solute.

The real interest in these membranes, however, arises when they are converted into the geometry of synthetic vesicles or 'liquid surfactant membranes'⁴. This geometry consists of small drops of solution coated with the liquid membrane and suspended in a solution of different concentration. Because these bubbles have such a large area per volume, diffusion in and out of the bubbles is much faster than is possible with classical membrane geometries. Separation has been achieved on an industrial scale, but the selectivity of these separations was controlled by solubility differences alone.

Bubbles coated with membranes containing carriers, like those in Table 1, have three advantages over those based on solubility alone: they can concentrate as well as separate a given solute; they have inherently much greater selectivity; and they are applicable to a much wider variety of chemical systems. These advantages accrue from the specific chemical reactions between carrier and solute. Because these reactions include a much wider spectrum of effects than solubilities do, a much greater variety of phenomena are possible.

An example of the properties of the bubbles coated with membranes containing carriers is shown in Fig. 2. In this experiment, 10 ml of 0.1 M NaOH was prepared. The membranes were made of light mineral oil containing 4 weight % trioctylamine and 1 weight % sorbitol monooleate. These bubbles were added, whilst stirring, to 100 ml of solution containing 100 p.p.m. chromium at pH = 1.6. The concentration of chromium in the bubbles rose from an initial value of zero, past the concentration in the bulk solution, to a value of 900 p.p.m. after 4 min, and the chromium concentration in the bulk solution decreased correspondingly. The very rapid uptake with very little amine provides an intriguing alternative to conventional ion exchange.

We have shown here how the membrane mechanisms postulated in biology can be generalised to produce a variety of chemically well defined analogues to living membranes. Although we have reported only data for inorganic ions, these principles can be extended to other solutes, including antibiotics, detergents, and amino acids. When these transport systems are used in conjunction with the technology developed for liquid surfactant membranes, separations of considerable

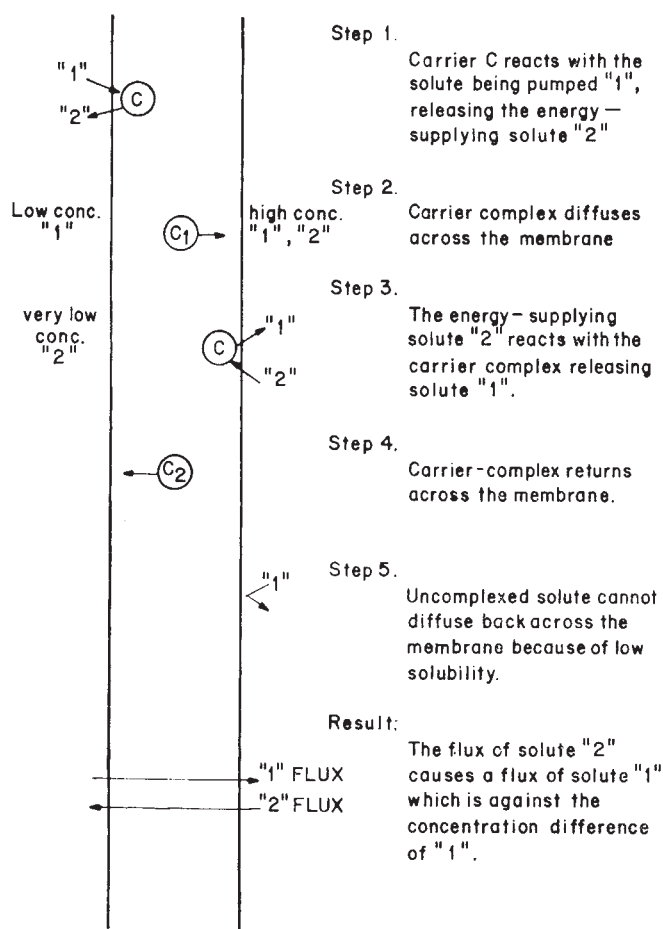


Fig. 2 Concentration of chromium against its concentration gradient using liquid surfactant membranes. *a*, In bubbles; *b*, in bulk solution.

practical potential are obtained.

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Removal of ozone from the atmosphere by soil and vegetation

OZONE is the principal constituent of the photochemical smog that plagues many cities in the United States. Produced by the action of sunlight on the hydrocarbons and oxides of nitrogen emitted by vehicles and industry, concentrations of ozone greater than 25×10^{11} molecules cm^{-3} (1×10^{11} molecules $\text{cm}^{-3} = 0.4$ parts per hundred million $= 8 \mu\text{g m}^{-3}$), used as evidence of photochemical smog¹, have been observed in Los Angeles for more than two decades². Because of lower air temperatures, less sunshine and fewer vehicles, photochemical pollution was considered unlikely to occur in Western Europe but concentrations indicative of photochemical smog have now been reported from Germany³, the Netherlands^{4,5} and southern England^{6,7} on calm, sunny days. High concentrations of ozone cause respiratory difficulties in humans⁸ and damage many plants⁹ including crops¹⁰.

The Earth's surface acts as a sink for ozone produced in the upper atmosphere¹¹. The rate of removal of ozone varies with the surface: water, snow, grass, and a juniper bush remove it increasingly quickly^{12,13}. Since soil bare of vegetation also removes ozone from the atmosphere at rates similar to those previously observed over land¹⁴, the role of vegetation is not clear. Laboratory studies with individual plants have indicated that vegetation removes ozone from the air when the stomata are open¹⁵⁻¹⁷.

We have examined the role of vegetation and soil as sinks for atmospheric ozone in the field. For this we constructed a mathematical simulator of ozone removal by vegetation and soil. In the laboratory, the rate at which bean plants removed ozone from the air corresponded to a resistance to ozone removal about the same as that encountered by water leaving the leaves through the stomatal pores¹⁵. After taking into account the different rates of diffusion of water vapour and ozone, we obtained similar results for young maize plants. The resistances to ozone removal and evaporation were 271 and 270 s cm^{-1} respectively in the dark and only 3 and 4 s cm^{-1} respectively in the light. This similarity of resistance to ozone removal and transpiration of water implies that at the cell walls beneath the stomata, where the air is saturated with water, the concentration of ozone must be essentially zero. Thus ventilation and

stomata alone control ozone removal by a leaf. This inference has suggested that simulators of evaporation can be modified to calculate the ozone removal by vegetation¹⁸.

Our simulator of ozone removal is based on a simulator of evaporation^{19,20}. The salient features of the simulator of evaporation are its conception of evaporation as an energy exchange process, the predominance of vertical exchange between the canopy and air above over horizontal exchange, the division of the canopy of leaves into strata, the use of stomatal and atmospheric resistances to evaporation, and the use of boundary conditions of temperature and humidity above and below the leaf canopy. The exchange of ozone can be compared with the flow of a direct electrical current through a series of resistances. This clarifies the concept of exchange and simplifies the algebra. The maize canopy may be depicted with eight strata of leaves and a basal stratum of stems.

The flux of ozone into each stratum of leaves or into the soil is opposed by vertical resistances to exchange from one stratum to the next, and within each stratum the exchange is opposed by the resistances of the boundary layer and stomata. The vertical transfer resistances and boundary layer resistances are the same as encountered by sensible heat or vapour transfer, and the stomatal resistance only needs to be modified for the different rates of diffusion of ozone and water vapour. Using the rules of electrical currents, as shown elsewhere¹⁸⁻²¹, we can relate the products of the fluxes of ozone into the leaves and soil, and the resistance to these fluxes to the differences in concentration of ozone at the canopy top and near the ground. Thus, the simulator enables us to calculate the ozone removal by soil and vegetation from the concentration of ozone at the top and bottom of the canopy if we know the vertical transfer resistances, the resistances to removal by the stomata and leaf boundary layer, and the leaf area distribution.

We first calculated the evaporation from measurements of temperature and humidity above a canopy of known leaf area per stratum, the temperature and humidity near the ground, the radiation absorbed by the canopy, the stomatal

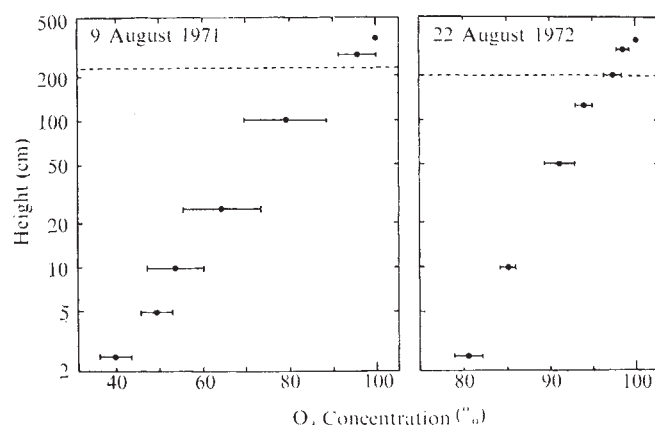


Fig. 1 Relation between ozone concentration and logarithm of height within and above a maize field. The concentration is presented as a percentage of that at 350 cm: 11×10^{11} mol cm^{-3} in 1971 and 31×10^{11} mol cm^{-3} in 1972. The dashed line indicates the height of the maize and the bars denote twice the standard error of the mean of four observations in 1971 and eight observations in 1972. Ozone concentrations were measured with a model 724-2 Mast (Mast Development Company, Davenport, Iowa) ozone meter fitted with a trap for sulphur dioxide. The meter was suspended from a vertical tower, so that it could be rapidly moved between pre-set heights from 2.5 to 350 cm above the soil. Each observation at a particular height was quickly compared with the concentration at 350 cm. Concentrations at seven heights were usually measured during a 20 to 30 min sampling period.

Table 1 Calculated removal of ozone by maize and soil

	August 9, 1971					August 22, 1972				
	Time (est.)									
	1330-1415	1415-1500	1500-1530	1630-1730	1730-1830	1500-1530	1530-1555	1630-1650	1715-1740	1740-1800
Ozone concentration* ($\times 10^{11}$ molecules cm^{-3})	9	9	11	12	10	31	34	36	32	30
Stomatal resistance† (s cm^{-1})	4	4	6	12	25	4	4	7	8	11
Ozone removal ($\times 10^{11}$ molecules cm^{-2} s $^{-1}$)										
(a) Foliage	8	7	5	4	2	27	24	16	13	9
(b) Soil (crop present)	6	6	6	8	4	7	8	8	6	9
(c) Total	14	13	12	12	6	34	32	24	19	18
(d) Bare soil‡	8	8	10	18	11	11	11	13	11	14
Soil and air layer§ resistance (s cm^{-1})	0.5	0.5	0.8	0.6	1	4	3	3	5	3

* At canopy top.

† Mean of two leaves per stratum for all strata.

‡ Assuming no crop present.

§ Layer 2.5 cm thick above soil.

and boundary layer resistances within each stratum and the vertical diffusivities within the canopy. We then compared the evaporation calculated by the simulator with actual observations of evaporation from a portion of the crop growing in a pair of weighing lysimeters.

Our test site was a 1 hectare field of a cultivar of *Zea mays* at the Lockwood Farm, Hamden, Connecticut. The maize was planted in mid-May to give a final population of 75,000 or 93,000 plants per hectare and a leaf area index (LAI) of 4.3 and 3.0 in 1971 and 1972 respectively. On August 9, 1971 and August 22, 1972, both clear sunny days with abundant ozone throughout the afternoon, the ozone concentration, net radiation, temperature, humidity, ventilation, stomatal resistance and evaporation were measured between 1330h and 1930h Eastern Standard Time (EST).

August 9, 1971 was slightly warmer and drier than August 22, 1972, and the combination of days and diurnal changes provided a range of weather conditions. The concentration of ozone varied markedly between the two days but not during a particular sampling period. The mean ozone concentration at 350 cm was 11×10^{11} molecules cm^{-3} in 1971 compared with 31×10^{11} molecules cm^{-3} in 1972; the decrease in concentration of ozone with height (Fig. 1) indicated that ozone was diffusing downwards into the canopy and soil. The comparison of evaporation from the lysimeters with that calculated from our observations of weather and plant factors for a range of conditions (Fig. 2; data obtainable from authors) showed that our estimates were realistic. We had to adopt the same rules as in ref. 24 to calculate the vertical resistances within the canopy and avoid impossibly rapid evaporation from the soil.

Assured that the vertical, boundary and stomatal resistances to vapour exchange in the different strata were realistic, we used them in the simulator of ozone removal. The simulated profiles of ozone concentration within the leaf canopies were then compared with the actual profiles measured in the field (Fig. 3). The good agreement gave us confidence in the simulator of ozone removal.

The calculated removal of ozone by the foliage plus soil at five times in both 1971 and 1972 is shown in Table 1. The calculated removal by the foliage was markedly affected both by the ozone concentration and by the aperture of the stomata. The flux of ozone into the soil beneath the maize was similar to that measured earlier¹⁴ over bare soil on several days when the ozone concentration was about 12×10^{11} molecules cm^{-3} .

We also calculated the removal of ozone by bare soil

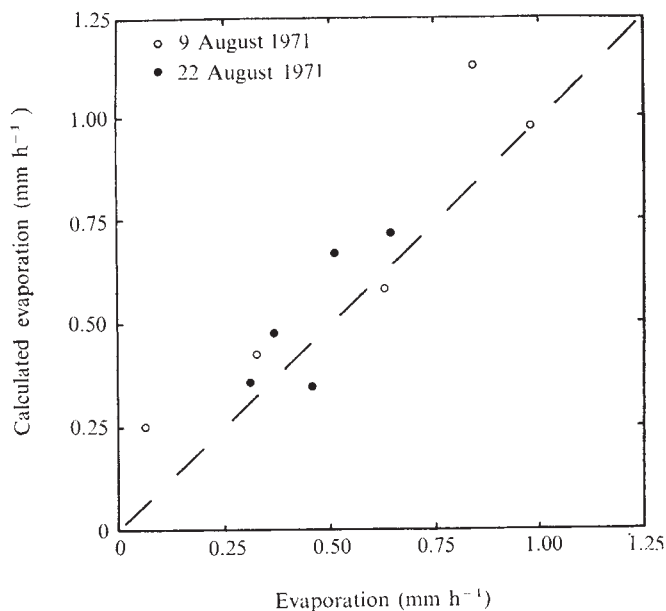


Fig. 2 Comparison of calculated and measured evaporation. To calculate evaporation, the net radiation above the crop was measured continuously with a Beckman and Whitley (San Carlos, California, Model 188-01) net radiometer. The coefficient of extinction of net radiation within the canopy was calculated from the leaf area and the logarithm of net radiation measured at five heights within the canopy with a portable net radiometer (Thorntwaite, Centerton, New Jersey, Model MNR540). The temperature and humidity at the canopy top (225 cm in 1971 and 200 cm in 1972) and at 10 cm above the soil were measured with a ventilated wet-bulb psychrometer (Bendix, Friez Instrument Division, Baltimore, Maryland, Model 566-2). The temperature, humidity, and net radiation within the canopy were measured near the beginning and end of each period. The horizontal wind was observed at four heights above and one height within the canopy with sensitive cup anemometers (Casella, London, Model T16112), and the mean windspeeds for the sampling period were then used to calculate the vertical transport coefficient, or diffusivity, at the canopy top. During each sampling period, the stomatal resistances of the upper and lower leaf surfaces of two leaves per stratum were measured with a ventilated diffusion porometer²². The resistances were measured on two plants growing in one of a pair of lysimeters²³ that measured the evaporation during each period. The lysimeter, ozone meter, and micrometeorological towers were approximately 7 m from the northerly edge and 150 m from the southwestern, leading edge of the field.

for the same concentrations of ozone but assuming that the canopy was not present. For this we used vertical resistances appropriate to bare soil¹⁴. The calculated removal of ozone by the soil had the crop been absent varied from 11 to 17×10^{11} molecules $\text{cm}^{-2} \text{s}^{-1}$ (Table 1). By difference, the additional removal of ozone by the foliage over that removed by bare soil was 0 to 23×10^{11} molecules $\text{cm}^{-2} \text{s}^{-1}$. Thus, even this more conservative estimate of the contribution of the foliage indicated that the crop increased the removal of ozone from the atmosphere while the stomata were open, but the decrease in ventilation by the crop decreased the flux of ozone into the soil and hence decreased the net impact of the crop. On the two occasions on August, 9 1971 when the calculation implied that the removal by bare soil would have been greater than by the crop and soil together, the stomata were closed, thus the foliage was removing little ozone, but it was slowing the ventilation and thereby slowing the removal of ozone by the soil.

The combined resistance of the soil and 2.5 cm air layer above it varied from 0.5 to 5 s cm^{-1} (Table 1), and was similar to the 2 s cm^{-1} observed previously¹⁴. Since the resistance of soil increases with moisture¹⁴, the variation in the resistance from 1971 to 1972 may simply reflect the greater amount of water in the soil in 1972. During the 7 d before measurement the rainfall was 3.5 mm in 1971 compared with 23.9 mm in 1972. Alternatively the differences may also reflect our inability to specify accurately the diffusivity in the air near the ground²⁴.

The calculations of ozone removal by the soil and maize are, however, reasonable, and clearly indicate that both soil and vegetation removed ozone when the stomata were open. It is known that in some species ozone closes the stomata^{25,26}, but our measurement of stomatal resistance were similar to those we had reported previously for maize^{24,27}, indicating that the maize stomata were not closed by the ozone. Clearly, however, closure of stomata or leaf injury by ozone will reduce the removal of ozone by vegetation.

Finally we determined whether a forest also cleanses ozone from the air. We chose a mixed hardwood and coniferous forest where the trees were about 25 m tall and had a leaf area index of about 3. We measured the concentration of ozone with a portable meter at a height of 0.3 m over a nearby lake and at seven stations within the forest along a 150 m line parallel to the prevailing southwest wind. Since the ozone concentration was similar all over the lake, only one station was established over the lake, 1 m from the shore. The meter was then moved

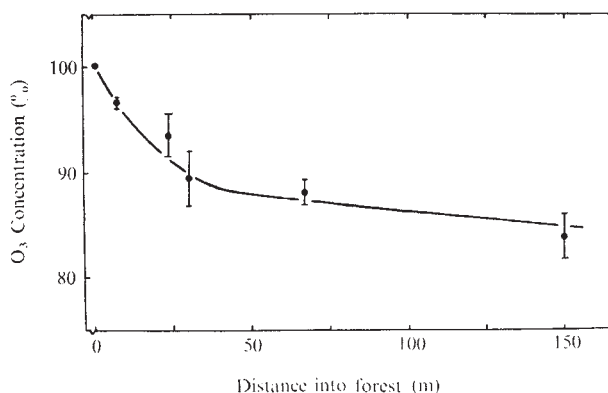


Fig. 4 Ozone concentrations measured at various distances from the shore of a lake into a forest. Concentrations are related to the 30×10^{11} molecules cm^{-3} at the shore. The bars denote twice the standard error of the mean of five observations between 1330 and 1850 h EST on August 23, 1972.

quickly between the stations, and the concentration observed for 1 to 2 min at each station. The decrease of ozone at stations within the forest (Fig. 4) indicated that the forest was removing ozone from the atmosphere, even when observation were made in the direction of the 450 cm s^{-1} wind.

Soil is a major sink for the carbon monoxide²⁸ and vegetation is an important sink for the sulphur dioxide²⁹ emitted into the atmosphere by man's activity. Since ozone is not emitted directly into the atmosphere, but is generated from other pollutants, a knowledge of its production in the atmosphere is required for a similar evaluation to be made for ozone. Recent estimates^{30,31} indicate an ozone production rate of nearly 10×10^{11} molecules $\text{cm}^{-2} \text{s}^{-1}$ in the lowest 2 km of the atmosphere. Clearly, a rate of removal or destruction of ozone of 6×10^{11} – 34×10^{11} molecules $\text{cm}^{-2} \text{s}^{-1}$ by soil and vegetation together could account fully for this estimated rate of production, and indicates that the soil and vegetation represent primary sinks for photochemically produced ozone.

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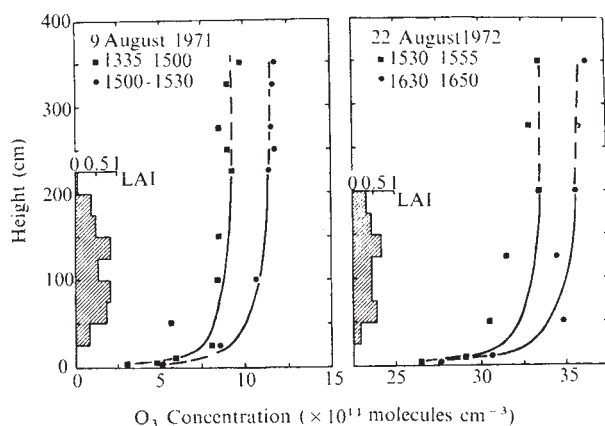


Fig. 3 Simulated (—) and observed (■, ●) concentrations of ozone in and above a maize field. The simulated profiles are extrapolated (—) above the canopy and below 10 cm. The leaf area index (LAI) of each stratum is also shown.

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How sea snakes may avoid the bends

MARINE snakes and turtles are probably the deepest diving reptiles. Sea snakes dive to maximum depths of at least 40 m with air in the lung¹. Pressure at this depth potentially produces the formation of N₂ bubbles in the blood and other tissues during decompression, yet sea snakes ascend to the surface rapidly and do not seem to be affected by caisson disease. Here I show that N₂ concentrations in the blood and tissues may remain much lower than that in the pulmonary blood because of a large venous shunt and a low but significant permeability of the skin to dissolved gas.

When a snake dives, the pressure in the lung increases, causing N₂ to move into the blood and from there into the tissues, tending toward equilibrium with pulmonary N₂. Because the N₂ concentration in seawater varies insignificantly with depth, diving creates a N₂ gradient between the blood and the seawater, and N₂ is lost at a rate dependent on the magnitude of this gradient and the resistance to diffusion through the skin. N₂ increases in the blood and tissues until the rate of gain from the lung equals the rate of loss through the skin. The N₂ content of the blood when equilibrium is reached is the maximum value that will occur at any particular depth. This value may be roughly estimated from the N₂ pressure in the lung, the diffusion coefficient of the skin, the cardiac output, and the degree of shunting of venous blood past the lung.

Because of the incompletely divided ventricle, shunting of venous blood from the right atrium to the systemic circuit (right to left shunt) may occur. Right to left shunting may also occur, in effect, if the blood in the pulmonary circuit does not fully equilibrate with the lung gas. For this discussion, it does not matter where right to left shunting occurs, in the heart or the lung. It is useful only to partition the systemic outflow into two fractions: one with the gas concentration equal to the venous return (that is, the shunted blood) and one having a gas concentration equal to that in the lung (that is, the effective pulmonary flow).

The net N₂ flow from the lung in ml min⁻¹ ($\dot{V}_L$) depends on the effective pulmonary blood flow in ml min⁻¹ ($\dot{Q}_L$) and the difference in N₂ concentration in ml ml⁻¹ between the blood functionally entering the lung (C_{sv}) and leaving it (C_{lv}).

$$\dot{V}_L = \dot{Q}_L (C_{lv} - C_{sv}) \quad (1)$$

The N₂ loss from the surfaces of the snake ($\dot{V}_s$) may be considered to depend on the diffusion coefficient D , and the gradient between the blood (C_{sa}) and the seawater. For this discussion, D is defined as the rate of gas movement through the entire gas permeable surface of a snake in response to a gradient of 1 atmosphere absolute (ATA) between the seawater and the arterial blood (ml min⁻¹ ATA⁻¹). Thus D varies with the size of the snake. This simplification permits conversion of the observed rate of extrapulmonary O₂ uptake to an estimate of N₂ diffusion out. A N₂ content (STPD) of 1 ml ml⁻¹ in blood is equivalent to a N₂ pressure of about 70 ATA at 30° C (ref. 2) and the N₂ pressure in seawater is about 0.78 ATA.

$$\dot{V}_s = D(70C_{sa} - 0.78) \quad (2)$$

At equilibrium, $\dot{V}_L = \dot{V}_s$.

$$\dot{Q}_L (C_{lv} - C_{sv}) = D(70C_{sa} - 0.78) \quad (3)$$

The N₂ concentration in the venous systemic blood (C_{sv}) is related to the systemic cardiac output ($\dot{Q}_a$), the rate of N₂ loss ($\dot{V}_s$), and the concentration in the arterial systemic blood (C_{sa}). Any heterogeneity in C_{sa} due to unequal distribution of shunted blood to the left and right aortic arches is ignored but, as will become evident, this does not greatly affect the results.

$$C_{sv} = C_{sa} - (\dot{V}_s / \dot{Q}_a) \quad (4)$$

Substituting (2) into (4)

$$C_{sv} = C_{sa} - [D(70C_{sa} - 0.78) / \dot{Q}_a] \quad (5)$$

Substituting (5) into (3) and solving for C_{sa}

$$C_{sa} = (0.78D(\dot{Q}_a - \dot{Q}_L) + \dot{Q}_a\dot{Q}_L C_{lv}) / (70D(\dot{Q}_a - \dot{Q}_L) + \dot{Q}_a\dot{Q}_L) \quad (6)$$

At equilibrium C_{sa} is maximal and is the highest N₂ concentration in the blood except in the pulmonary vein. The internal tissues may eventually equilibrate with the arterial blood so C_{sa} is important in determining the potential for N₂ bubble formation. Although C_{lv} may be much greater than C_{sa} , the small amount of N₂ in the pulmonary vein may be quickly diluted in the systemic circuit during decompression. C_{sa} increases with higher $\dot{Q}_L$ or lower D . If there is no N₂ loss ($D = 0$) or if no shunting occurs ($\dot{Q}_a = \dot{Q}_L$), the N₂ pressure in the blood and tissues approaches that in the lung ($C_{sa} = C_{lv}$).

It is possible to estimate equilibrium C_{sa} in a hypothetical 100 g sea snake diving to various depths (Fig. 1). Systemic cardiac output ($\dot{Q}_a$), estimated with the Fick principle from the arterial-venous O₂ difference of *Hydrophis belcheri* during spontaneous diving (R. S. S., and M. E. D. Webster, unpublished) and the rates of pulmonary and extrapulmonary O₂ uptake of a 100 g *Pelamis platurus*³, is assumed to be constant at 6 ml min⁻¹. The rate of cutaneous O₂ uptake is related directly to the O₂ gradient across the skin of *Pelamis platurus* (J. B. Graham, unpublished) and *Pseudemys scripta*⁴ which indicates that gas exchange is limited by diffusion rather than blood flow. At 30° C the O₂ uptake through the skin of *Pelamis platurus* is about 0.13 ml min⁻¹ with an O₂ gradient of one ATA (ref. 3). Because N₂ diffuses through tissue at about 55% of the rate of O₂ (ref. 5), the N₂ diffusion coefficient (D) becomes 0.07 ml min⁻¹ ATA⁻¹. Assuming 1 ml of blood holds 0.014 ml N₂ ATA⁻¹ (ref. 2), C_{lv} is calculated from the pulmonary N₂ pressure at depth. As not all of the O₂ absorbed from the lung is replaced by CO₂, N₂ accounts for about 95% of the lung volume after long breath holding (R. S. S., and M. E. D. Webster, unpublished).

The overall venous contribution to systemic cardiac output has been estimated to average 66% of the total in two sea snake species; about 80% of the blood in the left aortic arch and 50% of that in the right arch effectively bypasses the lung (R. S. S., and M. E. D. Webster, unpublished). If the blood N₂ threshold for bubble formation for cats (3.3 ATA at sea level)⁶ applies in sea snakes, decompression sickness cannot occur at depths less than about 50 m (Fig. 1). If no shunting occurred, the critical limit in the arterial blood would be reached at only 25 m,

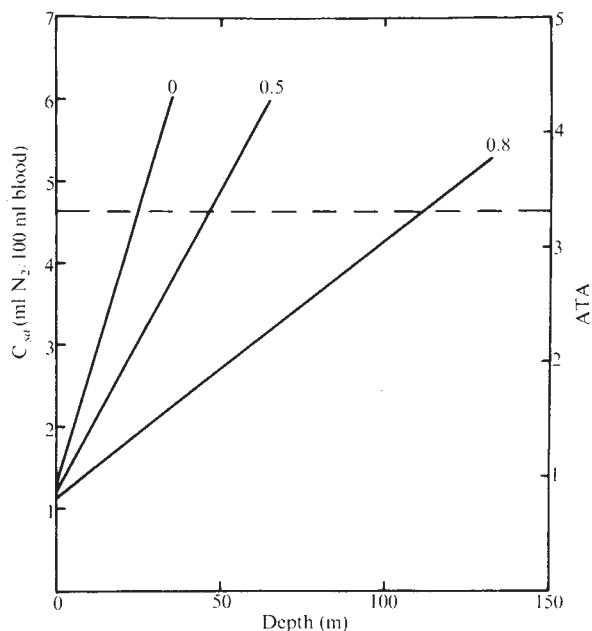


Fig. 1 Maximum blood N_2 concentration (C_{sa}) in a hypothetical 100 g sea snake when the rate of N_2 gain from the lung equals the rate of loss to the seawater at various depths. The effective shunting of venous blood past the lung is indicated as the fraction of systemic cardiac output. — — — Near the threshold for N_2 bubble formation in the blood of cats at sea level¹⁶.

a depth which produces caisson disease in humans following repeated breath-hold dives⁷. Lung volumes in diving *Pelamis platurus* (~ 9.5% of the body volume)⁸ and green turtles, *Chelonia mydas*, (~ 12% (ref. 9)) indicate that enough N_2 is available to saturate the tissues to dangerous levels. Equilibrium C_{sa} may never be reached, however, if voluntary dives are terminated before enough N_2 can be removed from the lung. Right to left shunting and cutaneous N_2 loss nevertheless decrease the risk of bubble formation during repeated or deep diving.

Because some degree of shunting may occur in other reptiles, it seems that sea snakes were preadapted for avoiding caisson disease when they invaded the ocean. Right to left shunting seems to be higher in sea snakes than in other reptiles breathing normally (R. S. S., and M. E. D. Webster, unpublished). Most reptiles show little or no intraventricular shunting when O_2 remains in the lung¹⁰. It is perhaps significant that all other measurements come from terrestrial or fresh water species which would not normally encounter great depths. Shunting should be measured in marine turtles which may dive to 290 m (ref. 11). Cutaneous gas exchange has been demonstrated in several species of turtles⁴. Berkson¹² observed that the arterial N_2 concentration in *Chelonia mydas* stabilises at values which are considerably less than those predicted by the pulmonary N_2 concentration at various ambient pressures. This occurred at pressures less than that required to compress the pulmonary gas into the rigid nonabsorptive air passages. Berkson suggested that shunting might account for this but did not consider cutaneous N_2 loss.

The mechanism described here may not be the only adaptation of sea snakes for avoiding caisson disease. It is possible that the poorly vascularised saccular portion of the lung limits N_2 absorption in the manner proposed for the tracheal and bronchial dead spaces in diving turtles¹² and mammals¹³. If this occurs in sea snakes, however, O_2 uptake would necessarily be inhibited and one would wonder why they dive with a considerable amount of air in the lung. Moreover, horizontal or vertical swimming might be difficult if the gas were retained only in the posterior saccular portion of the lung.

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On Allen's suggestion for long-distance translocation in phloem of plants

N. S. ALLEN has made a film of the streaming of cytoplasm in *Nitella*, in which she used laser light through chloroplast-free 'windows' in the walls of the giant alga cell to take a cine film under Nomarski optics. This film shows that the cytoplasm, which seems to flow steadily and spirally along the axis of the cell, contains undulating filaments that apparently provide the motive force for streaming. It is postulated¹ that endoplasmic filaments undulate in a sinusoidal fashion and thereby drive the cytoplasm. There is some supporting evidence that these filaments are branches originating from microfilament bundles which in turn are normally anchored and parallel to the fixed chloroplast rows. The waves passing along the semi-free microfilament branches cause them to act somewhat like beating flagella in other organisms.

Allen has suggested that some similar systems may be operating in sieve tubes in the phloem of plants. Two possible ways in which this might occur are given in Fig. 1. In Fig. 1a the microfilament material (m.f.m.) is shown attached to the plasma membrane and lateral walls of the sieve tubes. The swishing of these microfilaments would presumably continue through the sieve tube pores in sieve plates. In Fig. 1b (closer to the Allen proposal for *Nitella*) some fibrils persist as axial bundles from plate to plate, through the pores and lumina, though some may be anchored to the plates near the pores. Attached to these by one end only are loose branches of microfilaments which, it is postulated, can swish sinusoidally and hence propel sucrose solution linearly along channels through the lumen and sieve plate pores. We have carefully considered these suggestions in the light of the cine film study of D. R. Lee, D. S. F., and J. W. Costerton. Either model should produce an apparent mass flow with the profile of an activated diffusion², but model b fits more closely the evidence of the cine film study. Neither model will, however, account for a bimodal type of translocation whereby small pulses of sucrose precede the mass flow mode^{3,4}. We therefore propose a slight modification of Allen's hypothesis shown in Fig. 2, whereby the axial fibrils are thought to have flagella-like branches, but also to have hollow

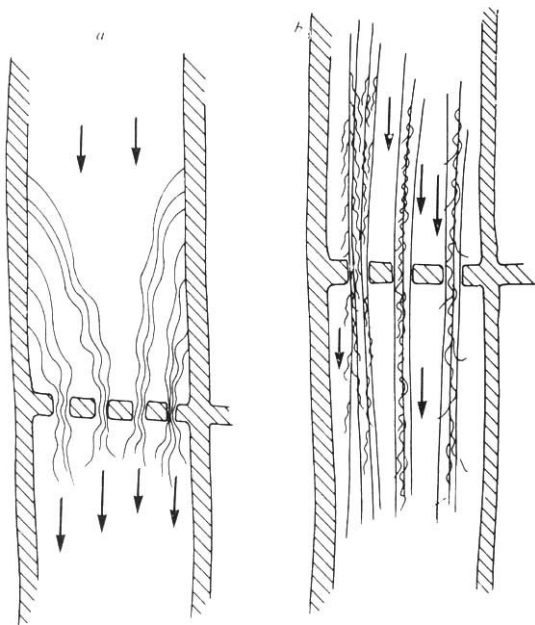


Fig. 1 Sieve tube and plate illustrating mass flow activated by swishing microfilament material (m.f.m.) attached *a*, to the sieve tube walls; *b*, to axial fibrils.

centres so that a small amount of sucrose can be moved ahead of the mass flow by a kind of microperistaltic movement. Such a model overcomes a difficulty in the proposals of D. S. F. whereby many parallel bundles 20–60 nm diameter would be required to operate in phase and in parallel through sieve plate pores. This new model agrees much more closely with the electron micrographs of Robidoux *et al.*⁵ and also with the freeze-etching work of Johnson⁶. It does require the existence of some transcellular material which an increasing number of investigators now believe to exist. But this axially oriented material could not be in the form of membrane-bound tubules of 5–0.5 μm in diameter, at least in *Heracleum*, for these would be clearly visible in living sieve tubes using Nomarski optics nor would a mass flow mode then be possible⁷.

The reasonable nature of these proposals can be demonstrated when Allen's hypothesis¹ is applied to calculate the probable order of magnitude of the energy dissipation in sieve tubes of *Heracleum*. We assume that the motive force causing flow operates throughout the length of the sieve tubes, that is, along the channels in the lumina and across the sieve-plate pores and we can write¹ the expression for the propulsive force per wavelength of a single filament as

$$F_p = 2\pi^2 b^2 V_m (C_N - C_T) / \lambda$$

where V_m is the measured velocity, about $3 \times 10^{-2} \text{ cm s}^{-1}$ (ref. 5); b is the amplitude of the vibration, about $2.3 \times 10^{-6} \text{ cm}$; λ is the wavelength, about $1.1 \times 10^{-5} \text{ cm}$.

The parameters b and λ are not based on *Heracleum*, for good micrographs by freeze-etching technique are not yet available for this plant. But Johnson⁶ has published electron micrographs of the sieve elements of *Nymphoides* using freeze-etching techniques. One of these (his Fig. 2) seems to show wave-like structures of repeating pattern suggestive of helical forms. Johnson has interpreted these as filaments, for they are more regular in pattern than the ramifications left between ice crystals and they appear as an array of dots. Tentatively assuming that these waveforms are the vestiges of filaments and that the pattern in *Nymphoides* would be reasonably similar to that in *Heracleum*, we have obtained the mean measurements given above.

C_N and C_T are the surface coefficients of resistance acting, respectively, normal and parallel to the axis of the tube. The

values of C_N and C_T for small fibrils can be calculated from the equations

$$C_N = 2C_T \text{ and } C_T = 2\pi \eta / (0.5 + \ln(r/\lambda))$$

where η , the viscosity, is taken as 2×10^{-2} poise (20% sucrose at 20°C) and r the radius of the filaments is taken as $5 \times 10^{-7} \text{ cm}$ (ref. 5). The numerical values of C_N and C_T are then 0.26 and 0.13 poise, respectively.

Hence $F_p = 1.7 \times 10^{-8}$ dyne per wavelength. The energy dissipation for mass flow will be $F_p \times V_m = 0.5 \times 10^{-9}$ erg per s per wavelength in a single filament. But in one sieve tube there are between 5×10^4 and 5×10^3 filaments in the cross section³ and 6.7×10^4 wavelengths per cm of filament. Hence in 1 cm of sieve tube the energy dissipation would be expected to be in the range 1.7 to 0.17 erg s⁻¹. These values are more conveniently expressed in terms of the rate of consumption of sucrose. For this we assume the number of individual sieve tubes per cm² of sieve tube cross section to be 3×10^5 (ref. 5) and take a value of 4×10^3 calorie per g of sucrose metabolised. Hence, for mass flow alone, the expected rate lies between 1.1×10^{-2} and $1.1 \times 10^{-3} \text{ g sucrose h}^{-1} \text{ cm}^{-2}$ which corresponds to only 1/9,000 to 1/90,000 of the sucrose carried by specific mass transfer, based on sieve tube cross section alone.

Of course, allowance must be made for the pulse flow moiety which seems also to be present. If such a pulse flow travelled by a microperistaltic process as suggested by D. S. F., then the number of transcellular fibrils conducting by this mode need only be perhaps 1/20 of the number previously calculated³ when it was envisaged that all the fibrils were potentially tubular in nature. In other words, we can consider that any one axial tubular fibril complex has at least 20 swishing filament tail units operating around it. Thus the sucrose consumption by this mode, and additional to that required for mass flow, would be about $2.3 \times 10^{-3} \text{ g h}^{-1} \text{ cm}^{-2}$.

Hence we conclude that the mechanisms operating either by undulating filaments alone or by filaments accompanying a

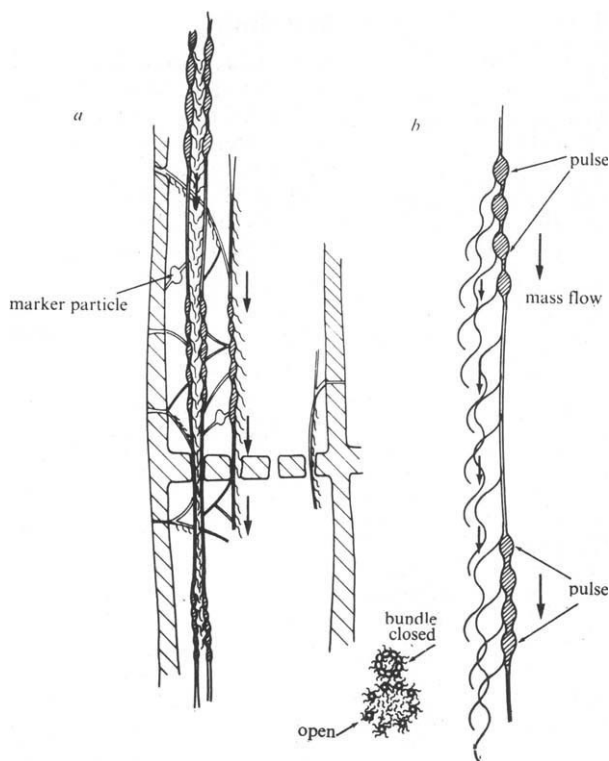


Fig. 2 Diagram of sieve tube and plate illustrating a bimodal system ('activated' mass flow + pulsatory flow) in which the m.f.m. are anchored to axial fibrillar bundles capable of microperistaltic action. *a*, Sieve tube; *b*, individual axial fibril with attached filaments (m.f.m. helices with branches in sinusoidal movement anchored to axial bundle).

microperistaltic moiety in the supporting fibrils are both energetically feasible and seem to be at least an order of magnitude lower than one using microperistalsis in 60-nm diameter tubules alone.

The strength of Allen's suggestion to us is that it provides a motive force through the lumen and sieve plate which assists rather than impedes flow past filamentous material, without invoking large membrane-bound tubules for which there is much less evidence than for transcellular fibrils or bundles of filaments. We hope that other workers in the field will be alerted to see whether further experimental evidence can be found to support it in other plants or to test it. Meanwhile it could be a very important concept in unravelling the problem of the mechanism of long distance sugar translocation in plants.

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kg⁻¹ water), to which ¹²⁵I-iothalamate was added as a volume marker³. This enabled accurate computation of the perfusion rate. Osmotic water permeability was calculated as previously described³. Perfusion was initiated within 90 min after hysterotomy. The length of the tubule exposed to the bathing medium was 400 μ m.

The osmotic water permeability after 80 min incubation was 2.48 μ l cm⁻² osmol⁻¹ min⁻¹. After the addition of vasopressin (2.13 mU ml⁻¹) to the medium bathing the tubule it rose to 11.98 μ l cm⁻² osmol⁻¹ min⁻¹. The values are of the same order of magnitude as those found in the isolated collecting tubule of the rabbit in the presence and absence of vasopressin³. The data are not strictly comparable, however, as the rabbit collecting tubules were obtained from the cortex and the nephron segment in the present study is from the medulla.

During the first hour of perfusion, the epithelial cells appeared cuboidal with barely visible intercellular spaces. The outer diameter of the tubule was 43 μ m and the inner diameter 21 μ m. After the addition of vasopressin, striking morphological changes occurred. These included cell swelling, vacuolisation and conspicuous dilatation of intercellular spaces (Fig. 1). These changes accompanied the increase in osmotic water permeability and indicated that outward net water movement occurs through intercellular channels as well as through the cell membranes. The structural changes are quite similar to those previously found in the isolated rabbit collecting tubule⁴.

Additional experiments are needed to substantiate the results of the present study. Nevertheless, our first data

Effect of vasopressin on the isolated human collecting duct

THE study of human kidney function has been largely indirect, based on clearance studies and interpretation of data from more direct approaches on other animal species. Extrapolation from one species to another, however, is not always justified and the need for accurate information on human kidney function is still great. Obviously, several ethical and practical factors severely limit the use of direct studies (such as micropuncture) on the human kidney.

If some portion of an intact human kidney was isolated *in vitro*, however, renal tubular transport could be studied directly. We have had the opportunity to carry out such a study which has involved isolation and microperfusion of human kidney tubules.

The kidney was from a male foetus 5.5 months old, which was aborted through hysterotomy. The indication for abortion was a D₁₁ trisomy¹ as diagnosed by karyotype analysis of cultured amniotic cells. The congenital defects included agenesis of olfactory lobes, pulmonary artery stenosis, ventricular septal defects and a single umbilical artery. The kidneys appeared normal in all respects.

The experimental procedure for the renal study was as follows. After removal of the left kidney, one slice was cut and immersed in chilled Ringer's solution. The slice was teased with small forceps and needles under a dissecting microscope². The tubules in the cortex were hard to separate. Eventually, a few fragments could be isolated from the medullary region and were identified as collecting ducts. One of them, after transfer to a special incubation chamber was successfully pump perfused using techniques previously described³. The bathing solution was a Ringer bicarbonate buffer with 5.5 mM glucose pH 7.4, gassed with a mixture of 95% O₂ and 5% CO₂ (ref. 2). The luminal fluid was a phosphate buffer containing 60 mM NaCl (125 mOsmol

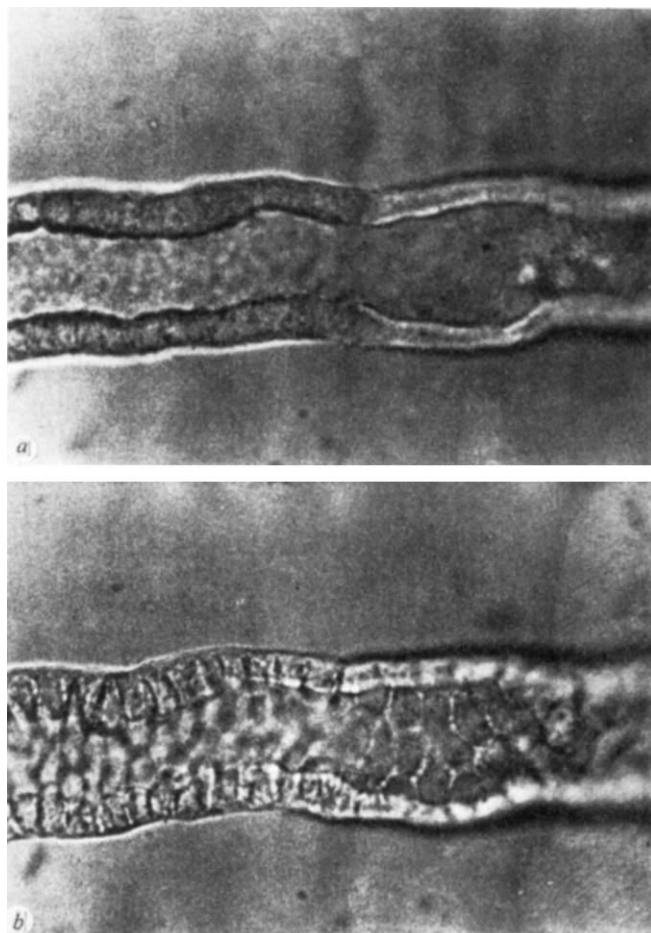


Fig. 1 Micrograph of living perfused human collecting duct. Focal plane at the central axis on the right and at apical surface of the cells on the left. *a*, Before vasopressin; *b*, after vasopressin ($\times 520$).

suggest that in man the collecting tubule receptors for vasopressin are well developed during prenatal life. It is likely therefore that the failure of the infant to excrete a concentrated urine cannot be attributed to a lack of end-organ responsiveness to antidiuretic hormone, as previously proposed⁵.

The inadequate renal concentrating ability in the newborn presumably reflects a poorly developed osmotic gradient in the medulla⁶.

The present study provides the first reported evidence that the function of the human nephron can be directly evaluated with the help of *in vitro* microperfusion techniques. Further work with similar material or with fresh, undamaged portions of surgically removed kidneys will be required to gain direct insight into renal tubular transport mechanisms in man.

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Mutagenicity of chromates in bacteria and its relevance to chromate carcinogenesis

THERE is abundant experimental evidence that certain chromium compounds are carcinogenic in animals^{1,2}. There are also epidemiological data which suggest that chromium compounds are carcinogenic in man^{3,4}. Calcium chromate especially, produces epithelial lung tumours both by intra-bronchial implantation¹ and sarcomata by intramuscular administration to rats³. In epidemiological studies Bidstrup and Case⁴ reported a significantly high lung cancer mortality in men who worked in chromate-producing factories, and concluded that the most likely explanation of this increased risk was due to an occupational carcinogen.

The mechanism of chromate carcinogenicity is not understood. But considerable evidence exists which suggests that chemical carcinogens are also mutagens although the converse is not necessarily true⁵. We have, therefore, tested the hypothesis that some chromium compounds, including a known carcinogen, are mutagenic. We have used a simple bacteriological spot-test mutagenicity assay⁶ which has convincingly demonstrated that simple hexavalent chromium salts of Na, K and Ca are indeed mutagenic under conditions where related heavy metal salts show no mutagenic activity. There is no evidence, however, that the highly soluble chromates such as Na and K used in this study are carcinogenic to experimental animals (ref. 7 and L. S. Levy, unpublished data), nor are there reports that they cause cancer in man.

The organisms used in this study were: *Escherichia coli* B/r WP2; WP2uvrA; WP2exrA, which differ in their sensitivity to and mutability by ultraviolet and ionising irradiation and a variety of alkylating and arylalkylating agents. All

strains require tryptophan for growth due to an *ochre* mutation in the *trpE* locus⁸.

E. coli K12(λ) CA165 (containing *SupB* (ref. 1) was used in conjunction with bacteriophage T4 *ochre* 427 for characterising the WP2 mutants obtained after chromate treatment⁹.

E. coli WP2 strains were grown overnight in nutrient broth at 37° C and diluted 1:50 in M9 medium containing 0.4% (w/v) glucose and 10 μg ml⁻¹ L-tryptophan. Cells were collected by filtration during mid-logarithmic phase, washed in M9 salts and resuspended at 5 × 10⁸ cells ml⁻¹ in M9 salts and kept on ice until used. For mutagenicity assays by the spot-test method 5 × 10⁷ bacteria were spread on agar plates containing M9 medium, 0.4% (w/v) casamino-acids and 1 μg ml⁻¹ L-tryptophan. Test compounds (Analar where available, otherwise General Purpose Reagent Grade, obtained from Hopkin and Williams, Romford, Essex, England) were dissolved in deionised water, filter-sterilised, and 5 μl applied to the centre of each plate. Pre-existing revertants were assayed on agar plates containing M9 medium, and never exceeded 2–3 colonies per five replicate plates. Plates were incubated at 37° C and the maximum yield of mutants (that is, revertants to tryptophan prototrophy) was obtained 4–5 d after plating.

Table 1 shows the results of such an assay using WP2 and three doses of Na₂CrO₄, K₂CrO₄, and CaCrO₄ respectively. Chromate-induced mutant colonies grew more slowly than the larger spontaneous colonies and at the higher doses a central zone of growth inhibition was visible. It is clear from the data presented in Table 1 that all three chromates were equally effective in inducing statistically significant increases (of the order of threefold) in the yield of prototrophic revertants over control levels. The values obtained are of course minimum estimates of the mutant yield since in this method of screening lethality cannot be assessed accurately.

Table 1 Reversion of *E. coli* WP2 (try⁻) to prototrophy after exposure to chromates*

Dose of compound, (μmol per plate)	Revertants per plate (mean of three plates ± s.d.)		
	Na ₂ CrO ₄ P†	K ₂ CrO ₄ P	CaCrO ₄ P
Control	37 ± 10	37 ± 10	37 ± 10
0.05	125 ± 8 < 0.001	114 ± 16 < 0.01	133 ± 26 < 0.01
0.10	105 ± 10 < 0.01	125 ± 7 < 0.001	99 ± 40 < 0.1
0.20	55 ± 6 < 0.1	127 ± 17 < 0.01	113 ± 34 < 0.05

* Spot-test method.

† P refers to probability in *t* test.

In order to confirm the results obtained using the spot-test method we performed a number of experiments in which bacteria in suspension were treated with Na₂CrO₄, after which the chromate was removed and mutation and lethality then assayed simultaneously (Fig. 1). In both *E. coli* WP2 and WP2uvrA the yield of induced revertants increased linearly with increasing chromate concentration, and there was no significant difference in mutability between the two strains. *E. coli* WP2uvrA was slightly more sensitive to the cytotoxic effect of Na₂CrO₄ than was WP2 (which is wild type with respect to excision repair), the 1/e doses being 2 mg ml⁻¹ and 2.7 mg ml⁻¹ respectively.

We obtained negative results with soluble salts of tungsten and molybdenum (the two metals closest to chromium in the periodic table) and also with a soluble trivalent chromium compound, Cr₂SO₄·K₂SO₄·2H₂O. Salts of zinc, cadmium and mercury were also tested and found to be inactive. From the present data, therefore, it seems that of the metals tested, only hexavalent chromium is mutagenic in this system, of which CaCrO₄ alone has been shown to be carcinogenic *in vivo*^{1,2}.

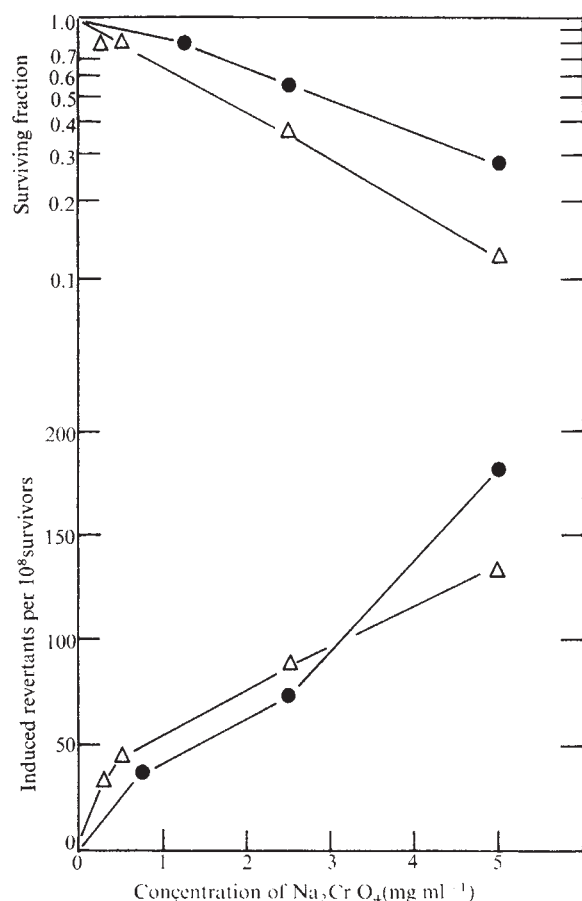


Fig. 1 Dose against survival and mutation of *E. coli* WP2 and *E. coli* WP2uvrA following treatment with Na₂CrO₄ in suspension culture. Bacteria were grown to mid-logarithmic phase (2×10^8 ml⁻¹) in M9 medium containing 10 µg ml⁻¹ L-tryptophan and collected by filtration and washed in M9 salts. Washed cells were resuspended in M9 salts containing 10 mM Mg²⁺ and 1 mM Ca²⁺ and treated with graded doses of Na₂CrO₄ for 2 h at 37° C. The treated bacteria were washed free of chromate by three cycles of centrifugation in ice-cold M9 salts and resuspended in M9 salts. For assay of viable counts, triplicate 0.1 ml samples at appropriate dilution were plated. Reversion to tryptophan independence was assayed by plating quintuplicate 0.2 ml samples of undiluted bacteria (5×10^7 ml⁻¹) per plate. The plating medium for both viable-count and reversion assays contained 1.5% agar in M9 medium, 0.4% casamino acids, and 1 µg ml⁻¹ L-tryptophan. Viable counts were scored after 18 h at 37° C, and the full yield of revertants was scored after 4 d at 37° C. Pre-existing revertants were assayed on M9 medium plates. The frequency of induced mutations was calculated according to the formula of Sedgwick and Bridges¹⁵. Each point represents the mean number of colonies of three plates (viable counts) and five plates (mutation frequency). ●, WP2; △, WP2uvrA.

The absence of either the *exrA* repair pathway ('error-prone') or the *uvrA* repair pathway ('excision-repair') did not significantly modify the mutagenic response to chromate (Fig. 1 and Table 2) in this test system. We therefore conclude that chromates fall into that class of mutagens exemplified by hydroxylamine¹⁰, bisulphate¹¹ and ethyl methanesulphonate (EMS) (ref. 14 and E. M. Tarmy, personal communication) which exert their effect by directly modifying DNA bases in such a way that base-pair errors arise at subsequent cell divisions. They do not fall into the second category of mutagens, such as methyl methanesulphonate¹², ultraviolet irradiation¹³, 7-bromomethylbenz[a]anthracene⁹ and nitroquinoline-*N*-oxide¹⁴, whose mutagenicity can be drastically modified or even abolished by the presence or absence of DNA repair processes.

Table 2 Mutagenicity of K₂CrO₄ in three DNA-repair strains of *E. coli* WP2*

Bacterial strain	Revertants per plate (mean of three plates ± s.d.)			<i>P</i>
	Control	Treated (0.05 μmol K ₂ CrO ₄ per plate)		
WP2	Exp. (a) 37 ± 10	114 ± 16		< 0.01
	Exp. (b) 28 ± 6	155 ± 20		< 0.001
WP2 _{exrA}	Exp. (a) 22 ± 8	65 ± 5		< 0.001
	Exp. (b) 30 ± 9	70 ± 6		< 0.001
WP2 _{uvrA}	1 Exp. 37 ± 7	74 ± 11		< 0.02

* Spot-test method.

We investigated the specificity of the postulated base-pairing error by determining the ratio of true revertants (that is, structural reversions at the *trpE* locus) to *ochre*-suppressor-containing revertants in a population of chromate-induced mutants (Table 3). We found that in the chromate-treated group, 98% of revertants contained *ochre* suppressors compared with 45% in the untreated (spontaneous) group. The reversion from tryptophan auxotrophy to prototrophy in this system involves mutation at an *ochre* triplet, UAA (containing only AT base-pairs in DNA)⁸.

Table 3 Characterisation of *E. coli* WP2 revertants obtained after treatment with K₂CrO₄

	No. of revertants lysed by T4 <i>ochre</i> 427	No. of revertants not lysed by T4 <i>ochre</i> 427	Total no. of revertants tested
Spontaneous revertants (untreated)	9	11	20
Induced revertants (chromate treated)	98	2	100

100 revertant colonies arising from a K₂CrO₄-treated plate, and 20 revertant colonies from an untreated plate, were purified by repeated subculture on minimal medium agar, and overnight nutrient broth cultures were prepared. A lysate of bacteriophage T4 *ochre* 427 was prepared by growth in and lysis of *E. coli* K 12 CA 165 in M9 containing 1% lactose and 0.4% casamino acids. This lysate, after centrifugation, contained less than 0.04% revertant bacteriophage when assayed on *E. coli* WP2. 0.2 ml of each purified revertant culture was mixed with 0.1 ml T4 *ochre* 427 (2×10^4 PFU ml⁻¹) in 0.6% agar at 46° C and poured on to plates containing nutrient agar supplemented with 1% glucose. *E. coli* WP2 which is not lysed by T4 *ochre* 427, and *E. coli* CA 165 which is, were used as controls¹⁶.

Since the treated population contained only 2% true reversions (of which some may well be spontaneous in origin), it is highly likely that chromate does not modify AT base-pairs, but specifically attacks GC base-pairs, causing GC to AT transitions at a subsequent round of DNA replication. In this respect, as well as in its mutagenicity in *Exr*⁻ bacteria, chromate closely resembles EMS, hydroxylamine and bisulphite, which have also been shown to specifically induced GC at AT transitions^{10,11}.

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Transepithelial potential difference in toad urinary bladder is not due to ionic diffusion

KOEFOED-JOHNSEN and Ussing proposed¹ that the transepithelial potential difference (PD) in frog skin was due to the sum of two diffusion potentials, one at the outside border due to sodium, and one at the inside due to potassium. Others have shown somewhat similar results in toad urinary bladder^{2,3}. Although there is evidence to support the notion that such a mechanism cannot account for the entire PD⁴⁻⁶, the hypothesis is still favoured by many investigators.

The hypothesis might be true if when the sodium pump is inhibited or stimulated, the same changes in PD occur

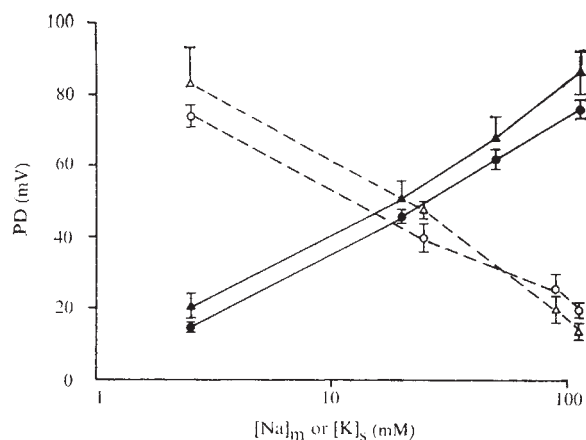


Fig. 1 Response of control bladders to ionic changes. The transepithelial potential is plotted against the mucosal sodium or serosal potassium (— — — —) concentration. ●, ○, Experiments performed with chloride as the major anion; ▲, △, Experiments done in sulphate media. In each case progressive Na for K substitutions were made in the appropriate medium and the resulting PD measured. Vertical lines indicate $\pm$ s.e.m.

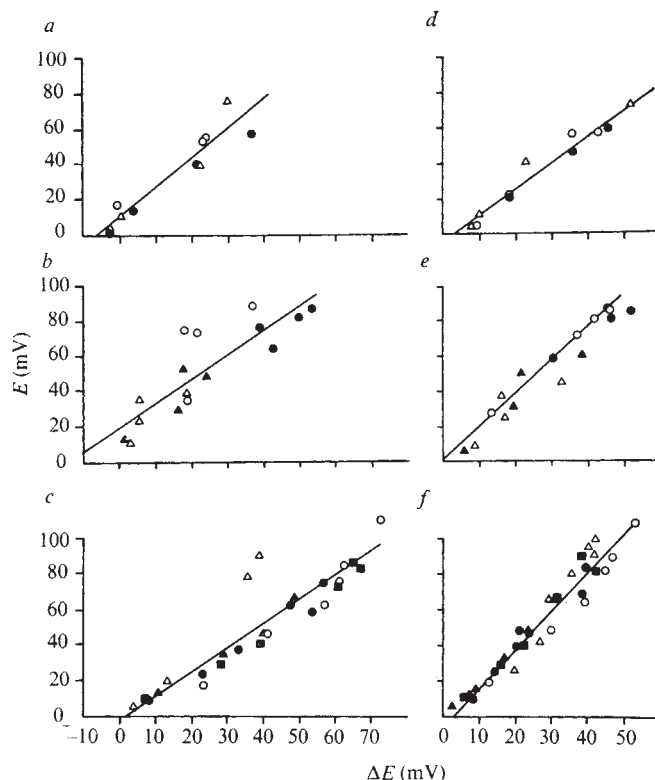


Fig. 2 Effect of inhibition of the transepithelial potential on the response to changes in ionic constituents. Each panel represents a series of studies on several bladders, and studies on a single bladder are shown for each panel as like symbols. *a* and *d* show the result of mucosal (*a*) and serosal (*d*) ionic changes in ouabain experiments. *b* and *e* show mucosal (*b*) and serosal (*e*) ionic changes in experiments in which the temperature was changed. *c* and *f* show mucosal (*c*) and serosal (*f*) ionic changes. *a*, $y = 1.65 (\pm 0.17) x + 10.3 (\pm 3.2)$, $r = 0.95$; *b*, in amiloride experiments, $y = 1.40 (\pm 0.21) x + 20.1 (\pm 5.8)$, $r = 0.88$; *c*, $y = 1.35 (\pm 0.12) x - 1.59 (\pm 5.16)$, $r = 0.91$; *d*, $y = 1.50 (\pm 0.10) x - 5.5 (\pm 2.9)$, $r = 0.98$; *e*, $y = 1.79 (\pm 0.13) x + 0.90 (\pm 4.1)$, $r = 0.97$; *f*, $y = 2.19 (\pm 0.11) x - 5.86 (\pm 3.28)$, $r = 0.97$.

when medium Na or K concentrations are changed as are seen in the control state, even though cell Na and K may have reached a new steady state value. That is, for example, whatever the cell K concentration, a tenfold change in serosal medium K would result in a more or less instantaneous tenfold change in the concentration ratio $K_{\text{cell}}/K_{\text{medium}}$, and the PD, a logarithmic function of the concentration ratio, should decrease by the same absolute amount.

Bladders were dissected from Colombian toads, (*Bufo marinus*), and none were used whose PD was less than 50 mV. The standard Ringer solution used contained (mmol l^{-1}): NaCl, 109; $CaCl_2$, 0.9; $NaHCO_3$, 2.4; KCl, 2.5; glucose, 5.5, and was stirred by means of a bubble lift with room air. When sulphate was used as the anion, the solution contained (mmol l^{-1}): Na_2SO_4 , 55; K_2SO_4 , 1.25; $CaSO_4$, 0.9; $NaHCO_3$, 2.4; glucose, 60. When step-wise changes in Na and K concentrations (carried out by K-for-Na substitutions) in the mucosal or serosal medium were made, results were similar to those previously described for this tissue^{2,3}, and were consistent with the interpretation that the mucosal barrier is more permeable to Na than to K, while the opposite is true for the serosal barrier (Fig. 1). Note also that there are only small differences between the results with Cl and those with SO_4^{2-} as the major anion. In all cases, the PD chosen was that seen 2 min after the previous solution was removed; each time a change was made, the chamber was washed three times with the new solution.

When the PD was reduced in seven experiments to 3 ± 1 mV with ouabain (10^{-3} M), however, the decrease in potential following reduction of mucosal Na to 2.4 mM was only 0.9 ± 1.1 mV, and that following the increase of serosal K to 111.5 was 2.3 ± 0.3 mV. Furthermore, when the ambient temperature was reduced to 5°C in two experiments (by means of circulation around the water-jacketed chamber of water from a constant temperature circulator), thus decreasing the PD to 0 and 4 mV, the changes in PD after reduction of mucosal Na to 2.4 mM were 0 and 0.5 mV, and the change after an increase in serosal K to 111.5 was 0 in both cases.

We next brought about a graded reduction in transepithelial PD by (1) progressive increases in the concentration of ouabain (added to the serosal solution only) from 10^{-5} to 10^{-3} M, (2) graded changes in the ambient temperature, and (3) progressive increases in the concentration of amiloride (added to the mucosal solution only, in concentrations ranging from 10^{-7} to 10^{-5} M).

Figure 2 shows the results plotted as ΔE , the change in potential after a solution change, against E , the PD obtained with Ringer on both sides just before the change. Note that essentially the same results were obtained with all inhibitors, and with either mucosal or serosal substitutions. Four further experiments with ouabain and two with amiloride were performed using SO_4^{2-} as the anion; equally strong correlation was found, with $r=0.98$ and 0.93 , respectively. Furthermore, if mucosal sodium was replaced by choline instead of potassium, identical results were obtained in two experiments with ouabain. None of these

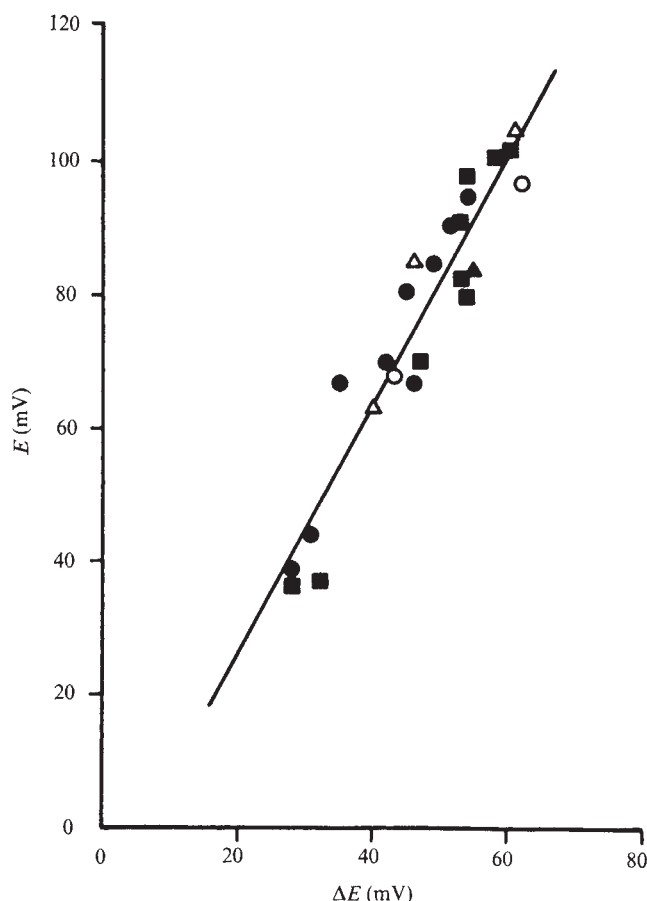


Fig. 3 Effect of stimulation of the transepithelial potential on the response to changes in serosal potassium. Data are plotted as in Fig. 2. The four rather low PD values are from two bladders whose PD decreased late in the experiment. In each bladder (shown by like symbols), serosal K was changed from 2.5 to 111.5 before and after the addition of vasopressin (25 mU ml^{-1}) to the serosal side. After the PD reached a new value, Ringer solution (with or without ADH) was replaced in the medium. $y = 1.96 (\pm 0.12) \times -15.5 (\pm 5.7) r = 0.96$.

Table 1 Transepithelial resistance

	Ouabain	Amiloride	Temperature change
Control	2305 ± 304	1493 ± 232	2270 ± 120
Experimental	2293 ± 322	2107 ± 231	3400 ± 209
<i>n</i>	5	4	5

Resistance (given in ohm cm^2) was measured either as the quotient of the open-circuit PD and the short-circuit current, or as $\Delta E/\Delta I$ when a 50 mV voltage step was applied for 20 s. These two techniques give identical results. In each case the experimental resistance shown is that measured at the highest concentration of ouabain or amiloride, or at the lowest temperature, usually 5° or 10°C , and all resistances are given as means $\pm$ s.e.m.

procedures resulted in a decrease in transepithelial resistance (Table 1), thus excluding the possibility that the observations could be due to a progressive decrease in tissue resistance as the baseline PD falls with inhibition of the Na pump. Finally, in experiments in which the transepithelial PD was stimulated with anti-diuretic hormone (ADH), the results of serosal K-Na substitution were identical with those seen with inhibition (Fig. 3).

Since these results are not compatible with the diffusion potential hypothesis, one explanation might be that the permeability characteristics of the mucosal and serosal barriers are in some way determined by pump activity, so that when the pump changes, the permselectivity changes. This requires, according to the results in Figs 2 and 3, however, that at every level of pump activity both mucosal and serosal permselectivity change *pari passu* with the pump. Furthermore, the procedures chosen for pump inhibition and stimulation are quite different in their sites and mechanisms of action, and the fact that nevertheless, both mucosal and serosal characteristics were affected in a similar manner make it unlikely that the resting potential in this and presumably similar epithelia is due to Nernst-like permeability barriers.

The obvious alternative is that the PD is due to the operation of a charge-separating or electrogenic pump, and that the apparent permselectivities deduced from changes in ionic constituents of the media in control preparations depend on the effects of the various ions on the pump itself. Since its characteristics cannot be rigorously defined by these experiments, however, further speculation seems unwarranted.

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Transmission abolished on a cholinergic synapse after injection of acetylcholinesterase into the presynaptic neurone

SINCE electron microscopy has enabled the identification of vesicles which appear concentrated at anatomically defined synaptic sites, a theory has been proposed that the transmitter released at the synapses is of vesicular origin. The

synaptic vesicles had a high content of acetylcholine (ACh)¹⁻³ and were ideal carriers of 'packets' of ACh released at the synapse, a necessary requirement for the quantal theory of transmission⁴.

Two hypotheses try to explain how the transmitter leaves the cells. One considers that the vesicles, having migrated to strategic presynaptic sites, fuse with the presynaptic plasma membrane and discharge their contents directly into the synaptic cleft⁵. Some electron microscope pictures of the vertebrate neuromuscular junction were interpreted as confirming such liberation of ACh by exocytosis⁶. According to the second hypothesis the ACh of the synaptic vesicles is released into the surrounding axoplasm before crossing the synaptic membrane.

So far, problems of transmitter storage and liberation have been approached mainly by anatomical and biochemical methods. We thought that a physiological approach was possible, taking advantage of the observation that ACh inside the vesicles is protected against the hydrolysing effect of acetylcholinesterase (AChE)^{1,3}, but that ACh in the cytoplasm would be rapidly hydrolysed by AChE.

By artificially introducing AChE into the presynaptic neurone in sufficient quantity to reach the presynaptic terminal, we were able to provide conclusive evidence that synaptic activity in the presence of intracellularly injected AChE is no longer possible. From the evidence presented here we assume that for ACh to act as a transmitter, it must be present free in the axoplasm (eventually released from synaptic vesicles) where it can be broken down by AChE.

In the buccal ganglion of *Aplysia*, two easily identifiable cholinergic interneurons make inhibitory synaptic connections with a number of postsynaptic cells situated close to each other in the same ganglion^{7,8}. After suitable dissection and attaching of the buccal ganglion in an experimental chamber constantly perfused with seawater, one interneurone (injected interneurone) was penetrated with a micropipette filled with purified 5% solution of electric eel AChE in seawater. (Identical results were obtained using AChE from Sigma (type V) or Worthington (type ECHP).) The purity of the Sigma preparation at least can be considered as very high, as when injected in the rabbit only the specific immune serum anti-AChE was found⁹. The injection was performed by an air pressure system similar to that used in a previous study¹⁰. The quantity of solution injected was not known. But, experiments made towards the end of the present study using AChE dissolved in tritiated water enabled the calculation of the intracellularly injected quantity as being about 1% of the volume of the cell soma. The propagation of the enzyme to the presynaptic site was followed with both light and electron microscopy, using an improved version of Koelle's histochemical method¹¹.

The injecting micropipette was also a microelectrode. The postsynaptic potential (PSP) produced by direct stimulation of the interneurone was recorded in one chosen postsynaptic neurone impaled with a double barrelled KCl-filled microelectrode. In some experiments, the second interneurone afferent to the same postsynaptic cell was also impaled (test interneurone) and the synaptic activity monitored. The postsynaptic potentials caused by direct stimulation of the injected and test interneurons were compared throughout the experiment to eliminate possible changes in the properties of the postsynaptic neurone during the relatively long experiments, especially the effect of changes of the membrane potential and of the intracellular ionic concentrations on the amplitude of the synaptic response. During measurement of the PSPs the membrane potential of the postsynaptic neurone was adjusted to -80 mV at which level the IPSP was reversed to a depolarising potential of several mV in amplitude which was more convenient for the experimental analysis.

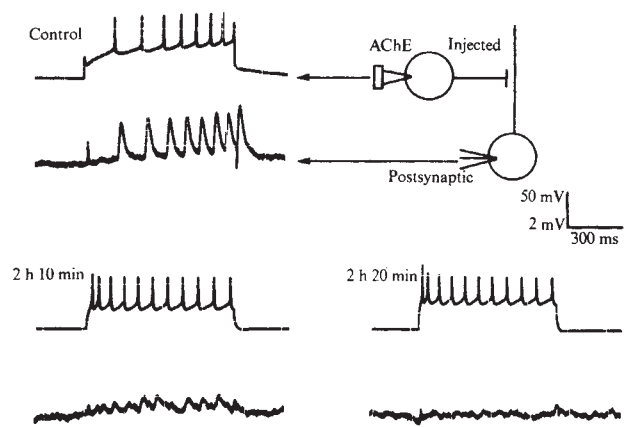


Fig. 1 Spike responses to direct stimulation of the AChE injected interneurone (upper traces) and the corresponding inverted IPSP in the postsynaptic cell at -80 mV membrane potential (lower trace). Control was taken 30 min after injection. At 2 h 20 min the depression of the PSPs was complete.

All experiments were performed at room temperature (20° – 22° C). After injection of AChE no significant change was observed in the respective amplitudes of the injected or test interneurone PSPs for about 2–2.5 h; then, in about 20 min or less, the PSP produced by stimulation of the injected interneurone decreased progressively and finally completely disappeared (Fig. 1). The amplitude of the test interneurone PSP remained within the normal range. The minimal time course of the depression of the PSP was identical whether the injected interneurone was previously firing at a high rate or whether its activity was completely abolished by hyperpolarisation.

In experiments in which presumably lesser amounts of AChE were injected, the injected neurone PSP decreased also but the changes in amplitude started after a longer time interval. Within 1 or 2 h an amplitude was reached which then remained relatively constant with respect to the test PSP or decreased slowly until the end of the experiment, that is for about 4 more hours.

It does not seem that the depression of synaptic transmission is caused by some modification in the size or con-

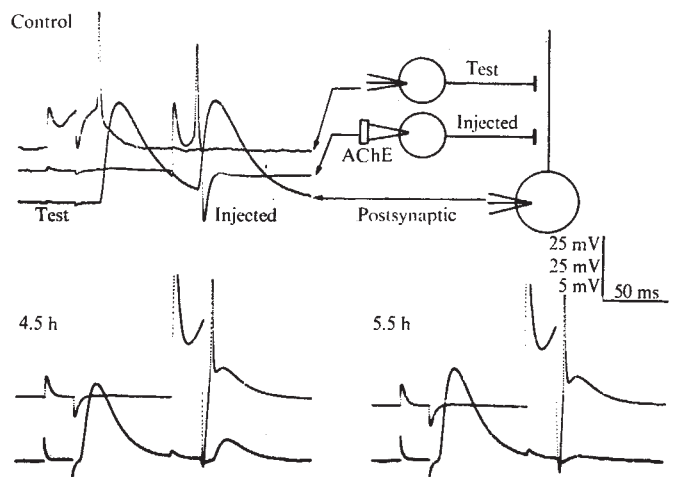


Fig. 2 Spike responses to direct stimulation of the injected and test interneurons and the corresponding inverted IPSPs in the postsynaptic cell at -80 mV. Traces of test spikes are omitted in the two lower recordings. Control was taken 30 min after AChE injection. The quantity of AChE was not sufficient to block the PSP due to the injected interneurone. The PSP corresponding to the test interneurone was not significantly changed.

duction of the spike, as the amplitude as well as the resting potential remained very much the same throughout the experiment (Fig. 2). Also the synaptic delay, in so far as it was detectable, remained constant. In addition the distance between the soma and the ending is short enough so that synaptic efficacy can be affected by modifying the somatic membrane potential by applied current (T. Shimahara and L. Tauc, unpublished) which leads one to suppose that membrane properties changing in the axonal part of the neurone would be detectable in the soma. Also the decrease of the PSP was continuous, without steps, indicating that there was no blocking of presynaptic spike on axonal branchings.

The injection of seawater alone or with addition of inactivated AChE in the same experimental conditions did not produce any change of synaptic efficacy in the injected cell. Contamination of our AChE preparation with proteases or any other enzymes seems highly unlikely. But, since no analysis was done, contamination causing the effect on synaptic activity observed here was ruled out by performing control experiments. Intracellular injection of mixtures of various proteases, especially trypsin and chymotrypsin, produced at higher concentrations marked modifications of the membrane potential and conductance, and of the spike of the injected cell. With concentrations too small to cause these changes in the properties of membranes no effect was detected on the level of the synapse.

Histochemical studies showed that AChE effectively propagates into the axon and in the axonal branchings in the neuropile (Fig. 3). The electron microscope (EM) pictures give additional evidence that AChE had reached the preterminal region of the injected axon. The black precipitate masks subcellular structures on the ending, but in some EM photographs, synaptic vesicles can be observed in spite of the abolition of transmission.

The distance from the soma of the studied terminal can

be estimated as <1 mm; the speed of propagation of the injected AChE was then about $200\text{--}300\ \mu\text{m h}^{-1}$, comparable with the slow rate of progression of AChE in the vagus nerve¹².

Unless there exists a quite improbable action of sub-products of ACh hydrolysis, or an unknown effect of AChE on molecules other than ACh, our results indicate that before its liberation, ACh has to be present in the axoplasm where it is exposed to the hydrolysing action of AChE. ACh is in the axoplasm because it is synthesised there; and possibly it comes from the synaptic vesicles. Vesicular ACh does not seem to be released directly into the synaptic cleft, a conclusion that can be deduced from the result observed here that the minimal time of AChE effect on active and inactive synapses is very much the same. Indeed, if ACh stored in the vesicles was released directly into the synaptic cleft a contrary result would be expected, because the vesicular ACh is protected against AChE action, and thus would remain available for longer. This theory is strengthened by the presence of quite considerable ACh stores in *Aplysia* cholinergic synapses, demonstrated by using hemicholinium-3, which need intensive and prolonged stimulation to become exhausted¹³, and also by a rather slow loss of vesicular ACh observed *in vitro*^{1,3} and *in vivo* when axoplasmic ACh of *Torpedo* was drained by intensive stimulation¹⁴.

It is interesting to note that the presence of axoplasmic releasable ACh demonstrated here in a form which can be attacked by AChE could also be explained by the existence of two pools of ACh, free and bound, the free pool containing the releasable ACh, a hypothesis which has been proposed by Dunant *et al.*¹⁵.

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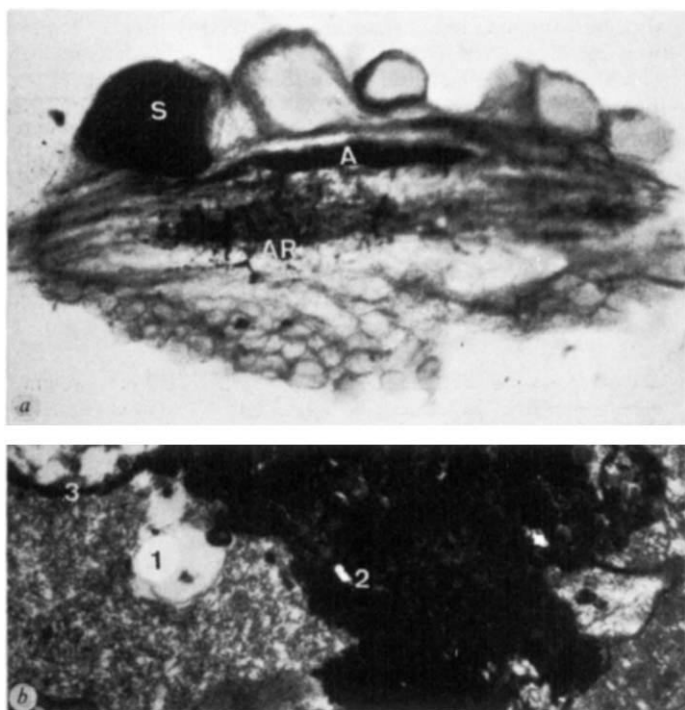


Fig. 3 *a*, Histochemical localisation of AChE injected into an interneurone of buccal ganglion of *Aplysia*. Thiocholine method. S, soma of BRI (right buccal ganglion, c.f. 7); A, axon; AR, axonal ramification. $\times 90$. *b*, Electron microscopic localisation of injected AChE detected in a preterminal region in the neuropile. Thiocholine method. $\times 23,000$. 1, nerve fibre without AChE injection; 2, preterminal region with injected ACh; 3, intercellular spaces with endogenous ACh activity.

Y chromosome effect on adult testis size

DURING the development of mammals with X and Y chromosomes the Y chromosome brings about the differentiation of the gonad primordia into testes¹. But apart from carrying the genetic determinants of maleness, little is known about its genetic constitution. In man, the gene for hairy tips to the ears shows Y linkage in at least some pedigrees². In mice there is evidence for a Y-linked histocompatibility locus^{3,4}, and for effects of the Y chromosome on the frequency of spermatozoa with abnormal heads⁵. Here we show that the size of the testes of adult mice is affected by factors on the Y chromosome.

Male mice of the CBA/FaCam strain are fully fertile, but have testes which are much smaller (90–110 mg) than those of males of most other strains^{6,7}. The differences remain significant when variation in bodyweight has been corrected for by regression analysis⁸. Small gonads have also been found in the J and Kw sublines of CBA^{7,9}. A comparison of CBA/FaCam mice with those of the A/Cam, SF/Cam and Peru strains showed that, after correction for variation in bodyweight, 87% of the observed variation was due to differences between the strains, 6% was due to litter effects and 7% to other environmental sources of variation. Reciprocal cross fostering of newborn mice between the CBA and Peru strains did not affect the size of the testes of adults of either genotype, showing that postnatal maternal effects were absent. A diallele analysis of simultaneous crosses between these three strains revealed significant general ($F_{6,321} = 383$) and specific ($F_{6,321} = 83$) combining abilities. There were significant differences between reciprocal crosses ($F_{6,321} = 48$). In all the crosses (see Table 1) F_1 males with CBA fathers had smaller testes than males from the reciprocal cross which had CBA mothers. This pattern of results is that expected for Y-linked factors but not for X-linked factors, or for direct maternal effects. Mice were bred from 18 further crosses between the CBA and SF strains. Table 2 summarises observations on the parental strains, reciprocal F_1 , F_2 and backcrosses, and on four F_3 and two F_4 crosses. All 11 stocks with Y chromosomes from the CBA strain had lower mean testis weights than the 11 stocks with a Y chromosome from the SF strain. Eleven $A \times CBA$ hybrid generations showed the same pattern of results. Table 2 indicates that the average effect of a CBA Y chromosome was to depress mean testis weight by 31 mg. More sophisticated maximum likelihood analyses⁸, however, estimated the Y effect as 24 mg, which represented 41% of the CBA-SF strain difference of 58 mg. In the F_4 generation the two lines which differed in Y chromosomes differed in mean testis weight by 24 mg. All genetic models which omitted a Y effect were much poorer fits than ones which included such an effect. The remainder of the strain difference was due to several (more than two) autosomal factors and to interactions between sex-chromosomes and autosomes.

The testes of CBA mice grew more slowly, at least after birth, than those of mice of other genotypes. The lower weight of testes from CBA mice was detectable well before puberty, and seemed to be due to differences in the length of the semi-

niferous tubules rather than in their diameter, or in the quantity of interstitial tissue present. CBA mice are fully fertile (personal observation and ref. 5) and had morphologically normal sperm⁵. Thus they were very different from C57BL/10J mice⁷, even though adult males of both strains had small testes. The C57 phenotype, which only developed after puberty, was not due to Y-linked genes⁷. The post-pubertal fall in testis weight was secondary to deficiencies in the production of androgens by interstitial cells. Spermatogenesis and fertility were consequently affected in C57 mice, as was the development of sex differences in several target organs of androgenic steroids¹⁰. Unlike the C57 mice, the CBA mice showed no signs of androgen deficiency and had normal spermatogenesis⁹ and a clear sex difference in kidney weight. CBA males were found to have smaller seminal vesicles than those of SF mice, but the inheritance of this difference was free from Y-linked effects⁸,

Table 2 The mean testis weights of CBA, SF and hybrid mice arranged in descending order

Stock	Y chromosome	Mean (mg)	s.e.	Sample size
F_1 (C) (S)	SF	165	2.0	66
BC (S) (CS)	SF	158	3.9	25
F_3 (SC.CS) (SC.CS)	SF	155	4.6	14
F_2 (SC) (CS)	SF	151	2.9	37
F_4 High	SF	146	4.3	9
BC (C) (CS)	SF	144	1.5	70
F_3 (CS.SF) (SC.CS)	SF	142	2.2	33
Strain SF (S) (S)	SF	140	1.9	75
BC (SC) (S)	SF	140	3.6	38
BC (CS) (S)	SF	138	2.5	55
F_2 (CS) (CS)	SF	136	2.8	62
BC (S) (SC)	CBA	132	3.6	38
F_1 (S) (C)	CBA	128	2.0	76
F_3 (CS.SF) (CS.SF)	CBA	125	6.7	8
F_4 Low	CBA	122	3.1	13
F_2 (CS) (SC)	CBA	121	3.0	61
BC (C) (SC)	CBA	121	1.8	69
F_3 (SC.CS) (CS.SF)	CBA	118	3.4	31
F_2 (SC) (SC)	CBA	110	2.9	61
BC (CS) (C)	CBA	107	2.0	64
BC (SC) (C)	CBA	101	1.8	68
Strain CBA (C) (C)	CBA	92	1.0	68

All measurements were made on mice aged eight weeks and were corrected to a bodyweight of 21 g by regression analysis. The letters in parentheses refer to the genetic origin of the hybrids. The first set define the female parent used in the cross and the second set define the male parent. Thus F_1 (C) (S) was the F_1 bred by crossing CBA females with SF males, and the F_2 (SC) (CS) mice were bred by crossing F_1 (S) (C) females with males from the reciprocal F_1 (C) (S) cross. The F_4 high mice were [(SC.CS) (CS.SF)] [(CS.SF) (SC.CS)] and the F_4 low mice were [(CS.SF) (SC.CS)] [(SC.CS) (CS.SF)]. BC, Backcross.

and was not causally related to variation in testis weight. Genetic variation affecting the sensitivity of seminal vesicles to testosterone is known^{11,12}.

The synteny of the CBA factors and the Y-linked histocompatibility locus is intriguing as factors concerned with the small testes of C57 mice seem to be linked to the H-2 histocompatibility locus on chromosome 17 (ref. 13).

The Y effect on gonadal development is large enough to be experimentally useful, yet does not produce infertility. Thus CBA mice, and hybrids derived from them, are excellent material for investigation of the function of the Y chromosome and for studies of the regulation of gonadal development¹⁴.

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Table 1 Mean paired testis weights (mg) for reciprocal F_1 males bred by crossing CBA mice to three other strains

Other parent	Reciprocal F_1		Difference $\pm$ s.e.	Sample size
	CBA mother	CBA father		
SF/Cam	167	135	$32 \pm 3^*$	47
Peru	144	104	41 ± 3	27
A/Cam	140	91	$49 \pm 4^\dagger$	23

All measurements were made on mice aged eight weeks, and were corrected to a bodyweight of 21 g by regression analysis.

* Data from Table 2 give a difference of 37 ± 3.9 ($N = 142$).

† Data collected on a different occasion showed a difference of 34 ± 3.5 ($N = 47$).

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Development and genetic analysis of *bithorax* phenocopies in *Drosophila*

PHENOCOPIES are developmental abnormalities which closely resemble known mutants, and can be induced experimentally¹. Phenocopies of a given mutant can be induced by various physicochemical agents, which share a unique and restricted effective period in development. In some instances it has been shown that the period of sensitivity to the agent coincides in turn with the effective period of the mutant itself^{2,4}. This is of special relevance because it places the phenomenon of the phenocopy in the same causal frame as that of gene action. Some of the morphogenetic mutants of *Drosophila* have been studied in clonal analysis and shown to act cell autonomously^{3,6}. We have investigated whether their phenocopies also result from events taking place at the cellular level.

In the present work phenocopies of mutants of the pseudoallelic *bithorax* system of *Drosophila* have been studied. Phenocopies of the mutant *bithorax*—one of the alleles—have been found following ether^{9,10} and temperature shocks¹¹ to embryos during the first 6 h of development. The genetic basis⁷ and developmental clonal parameters⁸ of these homoeotic mutants are known. Our main conclusions are: (1) phenocopies can be induced before the syncytial nuclei reach the cortex; (2) the effect is produced in individual cells which propagate their new state by cell heredity; (3) the genetic constitution of the egg, with respect to some alleles of the *bithorax* system, has no effect on its sensitivity to ether.

Eggs were collected, following a prelaying period, for 0.5 h on a filter paper sprayed with a yeast suspension. At different times after oviposition the filter paper was transferred for 10 min to a 50 cm³ chamber with a saturated ether atmosphere. The adults were scored under the binocular microscope and subsequently mounted in Euparal for microscopic examination.

In wild-type flies the frequency of *bithorax* phenocopies varies with developmental age (Fig. 1). Phenocopies appear in embryos treated less than 30 min after oviposition (before the syncytial nuclei reach the cortex¹²), their frequency increases to a maximum in embryos treated at 2–2.5 h (syncytial blastoderm) and decreases to zero in eggs treated after 5 h (following gastrulation). It is interesting

to note that the embryonic mortality which follows ether treatment is not correlated with the frequency of *bithorax* phenocopies (Fig. 1).

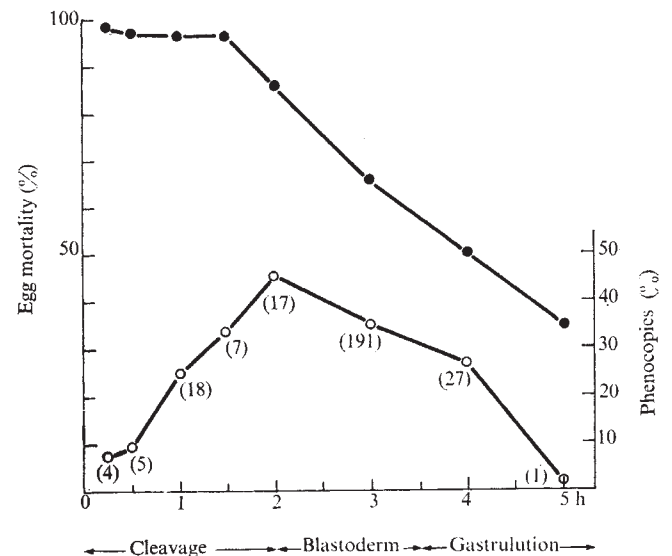


Fig. 1 Frequency of egg mortality (●) and of *bithorax* phenocopies (○) in wild-type individuals following ether treatment at different embryonic stages. The actual number of phenocopies is given in parentheses.

The phenotypes of the different pseudoallelic mutants of the *bithorax* system have been described in detail elsewhere⁷. *Bithorax* (*bx*) transforms the anterior and *post-bithorax* (*pbx*) the posterior metathorax, towards the corresponding regions of the mesothorax. Both regions are transformed in *Ultrabithorax* (*Ubx*) flies. A complete metathorax may appear instead of the normal mesothorax in *Contrabithorax* (*Cbx*) flies. Whereas the first two mutants are recessive and possibly correspond to structural loci, the last two are dominant and have been thought to represent operator mutations affecting the transcription of the entire *bithorax* system⁷.

In standard series of 3-h-old embryos, (experiments 1 and 2, Table 2) phenocopies of the different *bithorax* alleles appear with characteristic frequencies. A sample of 1,048 hemithoraces, corresponding to 524 phenocopy-carrying flies was studied. Phenocopies of *bx* in both dorsal and ventral (sternopleura) metathoracic regions appeared with a frequency of respectively 75% and 27% of the hemithoraces, and are usually the most frequent ones. Phenocopies of *pbx* are more difficult to identify, those with features of clear posterior wing, such as alula, appeared in 2% of the halteres. *Cbx* phenocopies—halter territories instead of wing structures in the mesothorax—were also found, but only in three cases (0.3%).

As previously observed⁹ phenocopies affecting both sides of the same thorax are more frequent than expected on the basis of independent events taking place on each side. This finding could indicate that the phenocopying agent produces some interference with the organisation of the embryo as a whole.

But, several observations suggest that phenocopies occur locally, affecting neighbouring cells in the embryo. Phenocopies are patchy, affecting different and discrete regions of the disk derivatives (see Fig. 2). In 728 hemithoraces, phenocopies embracing structures of both notum and wing represent 34% of the cases, those affecting only notum structures 11% and those affecting only wing structures 47%. In 54 cases (7%) we found independent spots in both notum and wing or in two separated regions of the wing.

Table 1 Frequency of *bithorax* phenocopies in different genotypes

Experiment	Cross	Adults	Phenocopies				
<i>n</i>	Female	Male	Genotype	<i>n</i>	<i>n</i>	%	ratio (bx)/TM1
1	<i>mwh jv</i>	↓ (Sevelen)	<i>mwh jv/+</i>	704	191	27	
2	<i>mwh jv</i>	<i>M(3)ⁱ⁵⁵/TM1</i>	<i>mwh jv/M(3)ⁱ⁵⁵</i>	2,112	438	21	0.8
			<i>mwh jv/TM1</i>	2,169	586	27	
3	<i>bx³e/TM1</i>	<i>sbd e</i>	<i>bx³e/sbd e</i>	302	100	33	0.9
			<i>TM1/sbd e</i>	269	99	37	
4	<i>pbx e/TM1</i>	<i>sbd e</i>	<i>pbx e/sbd e</i>	148	39	26	1.2
			<i>TM1/sbd e</i>	127	28	22	
5	<i>sbd bx³pbx e</i> <i>/TM1</i>	<i>Ubx¹/TM1</i>	<i>sbd bx pbx e/Ubx¹e</i>	325	0	0	
			<i>sbd bx pbx e/TM1</i>	508	148	29	
			<i>Ubx¹e/TM1</i>	530	127	24	
6	<i>Ubx¹e/TM1</i>	<i>sbd e</i>	<i>Ubx¹e/sbd e</i>	1,252	295	24	0.9
			<i>TM1/sbd e</i>	1,113	276	25	
7	<i>Ubx¹³⁰e/TM1</i>	<i>sbd e</i>	<i>Ubx¹³⁰e/sbd e</i>	324	168	50	1.7
			<i>TM1/sbd e</i>	327	98	30	
8	<i>Cbx e/TM1</i>	<i>sbd e</i>	<i>Cbx e/sbd e</i>	124	46	37	1.1
			<i>TM1/sbd e</i>	87	29	33	

Also, the shape of the phenocopy patches resembles that of mitotic recombination clones of, for example, *bx³/bx³* homozygous cells in a heterozygous *bx³/+* haltere background⁸. Also, phenocopy patches correspond in size to one or more recombination clones of *bx³/bx³* cells initiated in first instar larvae. Finally, the borders separating transformed from nontransformed cells in phenocopies are clear cut, as in recombination clones. All these facts taken together suggest that the process which produces phenocopies acts on cells or groups of neighbouring cells which retain their abnormal determination during subsequent development.

This hypothesis was tested in the following experiment. Eggs of the genetic constitution, *mwh jv/M(3)ⁱ⁵⁵* were treated with ether at an age of 3 h and the early second instar larvae (72 h) irradiated with 1,000 r. X rays. Mitotic recombination induced by X rays will give rise to non-Minute cells, homozygous *mwh jv* (two-cell marker mutants), which will overgrow their neighbouring Minute cells and build large marked clones¹³. Among 474 hemithoraces (from 358 flies) with phenocopies we found 38 cases of *mwh jv* clones in the dorsal metathorax in either the haltere or in the transformed wing territories. These clones never included both haltere and phenocopied wing cells. In 22 cases the borders of the clones ran for some way along the separation between transformed and nontransformed cells (Fig. 2). As expected these clones did not cross the compartment borders of the phenocopied wing or those homologous borders in the haltere¹³. These results demonstrate that phenocopied cells maintain in their clones the effects of the phenocopying agent after its removal.

The possible role played by the genetic constitution of the egg upon the frequency of phenocopies was tested in crosses of females heterozygous for *bithorax* mutants and a balancer chromosome (TM1, *Mé*, *sbd*) with wild-type males. In the progeny, flies carrying the mutations and flies carrying the balancer chromosome (internal controls) can be distinguished. As shown in Table 1 flies heterozygous for the recessive alleles *bx³* and *pbx* (experiments 3 and 4) or the doubly heterozygous flies (experiment 5) show similar frequencies of phenocopies to internal controls (TM1) and wild-type flies (experiments 1 and 2). Flies heterozygous for the dominant alleles *Ubx¹* and *Cbx* (experiments 6 and 8) behave in a similar way. However, *Ubx¹³⁰* heterozygous flies (experiment 7) show a much higher frequency of phenocopies than control flies. The extent and types of the phenocopies in flies heterozygous for *bithorax* alleles are similar to those in control individuals, mentioned above, with three exceptions: (1) *Ubx¹³⁰* flies show, together with a higher frequency, a higher expressivity in the transformation than flies of the other genotypes, including *Ubx¹*; (2) *Ubx* flies show *bx* phenocopies in

the haltere (Table 1), but also transformation of part of the mesothoracic haltere territory back into wing (9 out of 92 hemithoraces); (3) in *bx³ pbx/Ubx¹* flies, which are phenotypically *bithorax-postbithorax*, we did not find any transformation of either mesothoracic or metathoracic wing back into haltere.

These results indicate that neither the maternal genotype nor the allelic constitution of the cells, wild-type or heterozygous with respect to *bithorax* (with the exception of *Ubx¹³⁰*), affect the sensitivity of the cells to phenocopies. The possibility of phenocopying *Cbx* wings in wild-type flies, mesothoracic haltere in *Cbx* flies back into wing, but not mesothoracic or metathoracic wings of *bx³ pbx/Ubx¹* flies back into haltere, suggests that wing and haltere developments are not reciprocally reversible.

The gene products of the *bithorax* system are assumed to be active in normal flies in the metathoracic segment, preventing mesothoracic development, and inactive in the mesothorax⁷. Thus, we can suppress, by the action of phenocopying agents, the function of the *bithorax* genes in either the metathorax (*bx* and *pbx* phenocopies) or in the mesothorax (suppression of the *Cbx* phenotype), but we cannot produce a haltere development in flies defective for the *bithorax* genes. If we assume that ether interferes with segmental positional signals in the cortex¹⁴, metathoracic properties would appear in an otherwise mesothoracic segment (leading to *Cbx* phenocopies) or *vice versa* (*bx* and *pbx* phenocopies). This hypothesis is consistent with the fact that phenocopies can be obtained even before the migration of the syncytial nuclei into the cortex (Fig. 1). Thus, it is possible that *bx* and *pbx* phenocopies result from the repression of the *bithorax* system in the metathorax. It is therefore interesting that in heterozygous flies only *Ubx¹³⁰* leads to a higher frequency of phenocopies relative to controls. *Ubx¹³⁰*, a rearranged chromosome with one break point in the *bithorax* locus, could represent an operator-deficient mutant and so heterozygous *Ubx¹³⁰* flies will lack one of the two possible repressor binding sites.

We have seen that *bithorax* phenocopies induced in the blastoderm cells are faithfully transmitted in the progeny of individual cells. But, mitotic recombination in heterozygous cells for structural genes, for example, *bx³/+* haltere cells, induced any time during development, lead to clones of *bx³/bx³* cells which show growth and differentiation properties of the anterior wing⁸. Thus, phenocopies of *bithorax* must consist of a stable inactivation of the entire *bithorax* system, irrespective of the allelic state of their structural genes. This inactivation is reminiscent of the process of determination and indicates that both may take place at the cellular level. We hope that the combined studies of genetic variables and of their phenocopies will throw new light on the underlying mechanism of cell

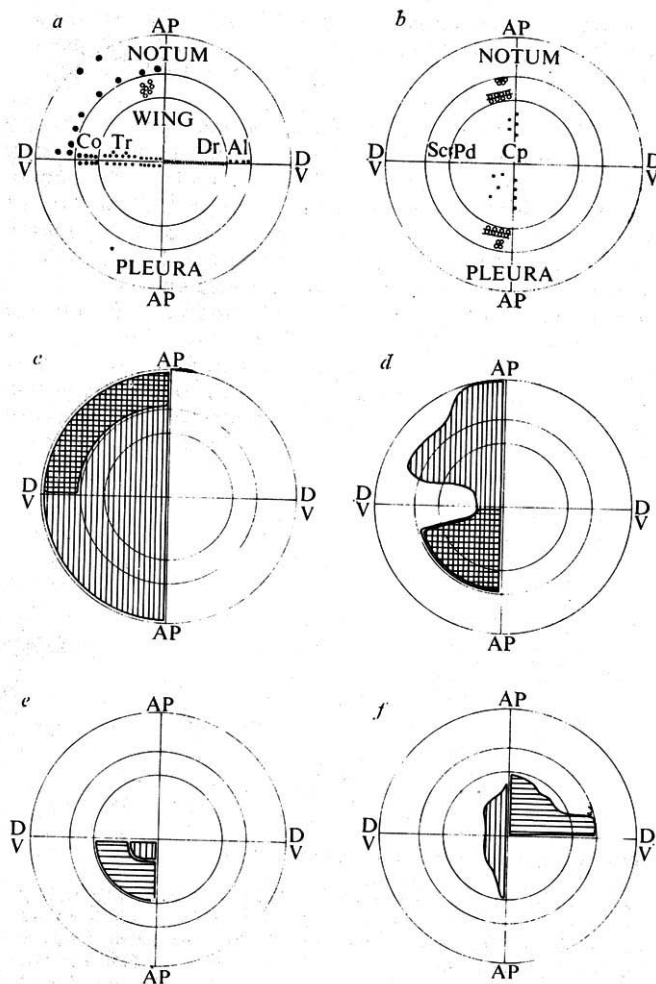


Fig. 2 a-f, Schematic representation of the adult dorsal mesothoracic (a) and metathoracic (b) disk derivatives with their region-characteristic landmarks (●, macrochaetes; ●, microchaetes; ○, sensillae). Regions in a, notum, pleura and wing with Co=Costa; Tr=Triple row; Dr=Double row; and Al=Alula. Regions in b, haltere with Sc=Scabellum; Pd=Pedicellum; and Cp=Capitellum. Quadrants correspond to developmental compartments (see ref. 13) defined by A/P: anterior-posterior, D/V: dorsal-ventral, notum wing and wing (or haltere) proximal-distal demarcation lines. c-f, Four examples of dorsal metathoracic disks showing the extent of 'bithorax' phenocopies, induced by ether in 3-h-old embryos, and of *mwh* *ju* *M*⁺ (non-Minute) recombinant clones initiated 72 h later. Phenocopied regions (hatched) contain mesothoracic structures (as in a) and clones (stippled) either mesothoracic (as in a) or metathoracic structures (as in b).

determination.

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Plasma binding of vitamin B₆ compounds

PYRIDOXINE, the major dietary form of vitamin B₆, is rapidly converted in the body to pyridoxal phosphate (pyridoxal-P), the coenzyme form. Pyridoxine has been shown to be phosphorylated in liver and brain homogenates¹ and in red cells², and is subsequently oxidised to pyridoxal-P. In the red cell pyridoxal-P is dephosphorylated, and pyridoxal is the form that is then released into plasma². But the endogenous form of B₆ found in plasma is mainly pyridoxal-P. This paper reports our findings on the ability of plasma proteins to bind these two circulating forms—pyridoxal and pyridoxal-P, and their dietary precursor, pyridoxine. We found that there were marked differences in the extent to which each B₆ compound was bound. These findings can be interpreted to explain part of the mechanism which regulates the passage of B₆ compounds into and out of the red cell.

Heparinised or clotted blood samples were obtained from normal human subjects. When heparinised plasma was compared with serum, no differences in the elution pattern of vitamin B₆ compounds were observed. The proteins were fractionated by gel filtration at 4° C on a 2.7×90 cm acrylic column (Wright Scientific, Kenley, Surrey, UK) of Sephadex G200, eluted with 0.05 M phosphate buffer pH 7.4 containing 0.154 M NaCl. The flow rate was 12 ml h⁻¹ and 130×4 ml fractions were collected. The vitamin B₆ compounds used were crystalline pyridoxal and pyridoxal-P (Sigma) and ³H-labelled pyridoxine (Radiochemical Centre) with specific activity adjusted to 62 mCi mmol⁻¹ by the addition of unlabelled pyridoxine (Sigma). Pyridoxal, and pyridoxal-P after acid hydrolysis to pyridoxal, were measured by microbiological assay using *Lactobacillus casei*³. ³H-pyridoxal, derived from red cell conversion of ³H-pyridoxine, was distinguished from ³H-pyridoxine by *L. casei* activity of the former². Protein was measured by absorbancy at 280 nm.

The gel filtration pattern of the vitamin B₆ compounds in the absence of plasma was first established (see Fig. 1). In these experiments each compound (1,000 ng in 2 ml saline) was eluted separately through the column and fractions were selected for microbiological assay or ³H-counting. In each case the vitamin B₆ eluted in a single symmetrical peak with maximum concentration in fractions 106-108 (free position).

Experiments were then carried out in which 1,000 ng (0.02 ml) of each vitamin B₆ compound was incubated separately with plasma (2 ml) for 30 min at 37° C and then subjected to gel filtration. Fractions were selected for microbiological assay, ³H-counting and protein measurement. Each compound eluted differently as shown by the representative set of experiments illustrated in Fig. 1. Pyridoxine added to plasma eluted in exactly the same fractions as pyridoxine in saline, demonstrating an absence

of binding to protein (Fig. 1a). In contrast, pyridoxal-P eluted almost entirely with the third protein peak (Fig. 1b) which consisted mainly of albumin, transferrin and 3.5S α -glycoprotein¹. In the case of pyridoxal, a significant proportion was associated with the same protein peak, but most eluted in the free position (Fig. 1c). Exactly the same pattern of elution was demonstrated for pyridoxal contained in samples of plasma separated after whole blood had been incubated with 500 ng ml⁻¹ of ³H-pyridoxine (Fig. 2) or pyridoxal.

The binding of the different B₆ compounds to plasma protein will be discussed in relation to our earlier findings on vitamin B₆ metabolism in blood². These demonstrated that the uptake of pyridoxine by red cells, after an initial rapid phase, slowly proceeded to completion as conversion of pyridoxine to its active forms took place in the red cells. This uptake and conversion were independent of the presence of plasma since the kinetics were identical whether the red cells were suspended in plasma or saline. It is therefore not surprising that we found no binding of pyridoxine by plasma proteins (Fig. 1a).

In contrast, pyridoxal-P is totally bound to protein (Fig. 1b) and pyridoxal seems to be partially bound (Figs 1c and 2). In relating these findings to our earlier work it is valuable to consider some observations made by Dempsey and Christensen⁵ in a study of B₆ binding to purified bovine serum albumin. These authors came to the conclusion that albumin has two specific binding sites for pyridoxal-P with relatively high association constants, but that in addition, both pyridoxal-P and pyridoxal bind less strongly to additional sites. If this also applies to human albumin in plasma, and we found that pyridoxal-P was completely eluted in the albumin-containing peak (Fig. 1b), then the strong

binding of pyridoxal-P by albumin would account for the inability of pyridoxal-P to enter the red cell from plasma, in contrast to its substantial uptake by red cells suspended in saline².

In view of the findings of Dempsey and Christensen⁵ and of the elution pattern we observed (Figs 1c and 2), it is probable that the pyridoxal-binding protein of human plasma is also albumin. Although only partial binding of pyridoxal is apparent from our results, the poor separation between bound and free peaks (Figs 1c and 2) is suggestive of dissociation of pyridoxal from albumin during gel filtration, in accordance with their weak binding⁵. The binding of pyridoxal in the fresh whole plasma may therefore have been complete. This binding had already been suggested by our studies on red cell metabolism of B₆, for we found that whether pyridoxal was derived from red cell conversion of pyridoxine or was added directly to blood, the proportion

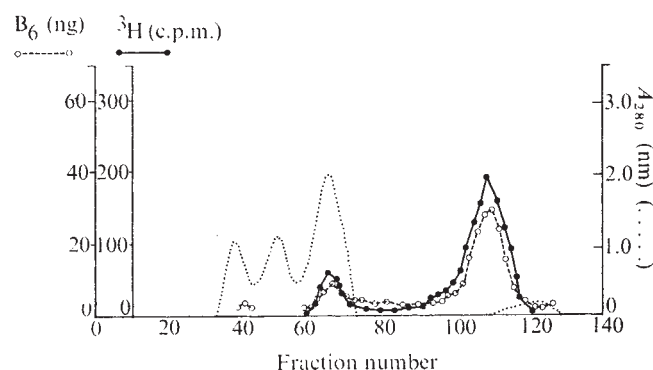


Fig. 2 Plasma protein binding of the pyridoxal originating from red cell conversion of pyridoxine added to whole blood *in vitro*. Heparinised blood was freshly obtained from a healthy subject and to 5.0 ml was added 2,500 ng ³H-pyridoxine (0.05 ml). The mixture was incubated for 2 h at 37° C in a shaking water bath kept in subdued light, after which the blood was centrifuged and the plasma removed. Gel filtration of 2.0 ml of the plasma (containing 520 ng ³H-pyridoxal as measured by microbiological assay and no residual ³H-pyridoxine) was carried out as described in the text for the other experiments, and the ³H-radioactivity (●—●), B₆ microbiological activity (○—○) and protein content (.....) of selected fractions were measured.

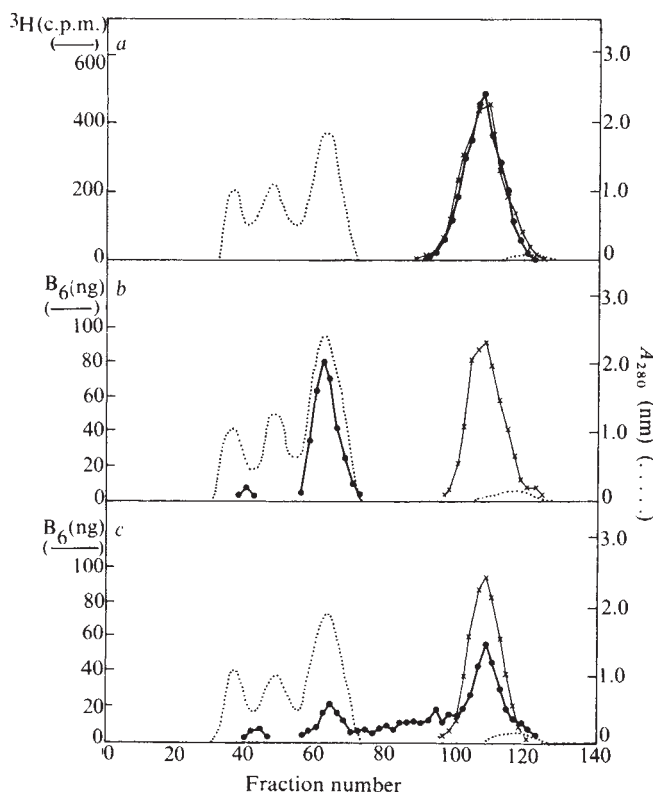


Fig. 1 Binding of a, pyridoxine; b, pyridoxal-P and c, pyridoxal to plasma protein. Gel filtration on Sephadex G200 of each compound added *in vitro* either to plasma (●—●) or to saline (×—×). Vitamin B₆ compounds detected either by ³H-radioactivity (pyridoxine) or microbiological assay (pyridoxal-P and pyridoxal). Protein determined by absorbancy at 280 nm (.....).

of pyridoxal in plasma was directly related to the amount of plasma present. In normal blood the proportion was approximately half that in the red cells, but if experimentally a constant volume of red cells was suspended in increasing saline dilutions of plasma, then the proportion of pyridoxal in the supernatant decreased; if plasma was totally replaced by saline or phosphate buffer, all the pyridoxal was accumulated in the red cells, confirming the work of Yamada and Tsuji⁶. It is probable therefore that the binding of pyridoxal to albumin in plasma plays an important part in the distribution of pyridoxal between the red cells and plasma.

Some additional mechanism must, however, be involved to explain the accumulation in red cells of pyridoxal when plasma is replaced by saline. Furthermore, the uptake of pyridoxal by red cells has been shown to be related to the volume of red cells, provided that the volume of plasma is kept constant (B. B. A., unpublished observations). These findings suggest that the distribution of pyridoxal between red cells and plasma may be controlled by competing binders in these compartments.

Further studies of the binding of vitamin B₆ compounds to plasma and to red cell components are being carried out with a variety of techniques in an attempt to confirm the identity of these binders and their significance.

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The effect of environmental lighting on porphyrin metabolism in the rat

THE direct action of light on the skin in porphyria is well documented. There is a specific spectral response in that light of wavelengths between 400 nm or 500-600 nm causes lesions through photodynamic action of porphyrin in the skin¹⁻⁴. Light may however affect abnormal porphyrin metabolism apart from any action on the skin. We noticed that skin lesions were provoked in porphyric rats, whose coats were shaved, by continuous irradiation by white light fluorescent tubes. Some porphyric animals, in which the coat was not shaved off as a control, excreted more porphyrin when under continuous light than other control animals under continuous dark. This implicated light as an environmental factor effecting porphyrin metabolism. Porphyrin excretion as a measure of abnormality, however, gave inconclusive results. Instead we have used the activity of hepatic δ -aminolaevulinic acid synthetase (ALA-S), the rate controlling enzyme in the haem biosynthetic pathway⁵, as an index of porphyrin abnormality. Normally, ALA-S activity is low, but where porphyria is present clinically or has been induced chemically in experimental animals, activity is raised⁶. We used 1, 4-dihydro-2, 4, 6-trimethyl pyridine-3, 5-dicarboxylate (DDC) to induce porphyria; this chemical, like some other porphyrinogenic agents, such as griseofulvin, probably acts through interfering with a negative feedback inducing mechanism involving haem⁷.

The ALA-S assay was performed on whole liver homogenates⁸ from Sprague-Dawley rats weighing 200-300 g. We first found that normal rats taken straight from the animal house, after life-long exposure to natural cycles of night and day, had a mean value for hepatic ALA-S activity of 26.09 nmol ALA per g wet weight per h (s.d. $\pm$ 7.68). The effect of exposure on six males and six females to continuous light for 11 d, or on six males and six females to continuous dark to 11 d, made no significant difference to ALA-S activity. This was not so, however, when porphyria was induced by DDC. Our final regime, which we shall call the light-dark-DDC regime, was as follows: days 1-8, food (FFG) (M) and water *ad libitum*; day 9, food and water *ad libitum* and DDC; day 10, food

Table 1 Effect of light on rat hepatic ALA-S activity

	Male	Female
Light + DDC	94.18 $\pm$ 51.16 (16) } *	263.50 $\pm$ 143.28 (16) } †
Dark + DDC	63.78 $\pm$ 33.82 (18) }	109.89 $\pm$ 77.62 (17) }

Mean ALA-S activity expressed in nmol per g wet weight per h $\pm$ 1 s.d. Numbers in brackets are the numbers of animals.
* Difference between light and dark treated, by *t* test, $0.05 > P > 0.02$.
† Difference between light and dark $P < 0.001$.

and water *ad libitum* and DDC; day 11, hepatic ALA-S assay. The rats were kept either in continuous light or dark for 11 d.

The light source was an array of eight parallel 20 W Atlas "white" fluorescent tubes, luminous flux approximately 1,000 lumens each, placed 25 cm above the cage floor containing six rats. The conditions of those animals kept in the dark were of photographic dark-room standard and the animals were only exposed to a few minutes of weak red light each day. The DDC was given by oesophageal tube as a 20% (w/v) suspension in arachis oil at a dose of 400 mg per kg body weight once daily on days 9 and 10.

In the light-dark-DDC regime, continuous light led to increased ALA-S activity compared with continuous darkness; this was more pronounced in the female than in the male (Table 1). Control experiments in which rats were given arachis oil, without DDC, showed no difference in ALA-S activity between light or dark treatment. A preliminary experiment of similar design using mice and griseofulvin as the porphyrinogenic drug, gave the same results as for DDC; increased ALA-S activity by light; but the remaining experiments we report are confined to the female rat and DDC.

To see if the stimulus for the above result might originate in the retina, we investigated the effect of orbitectomy. The difference in ALA-S activity previously found in intact animals was now abolished (Table 2a). Activity to hepatic ALA-S in the light-treated orbitectomised group, however, differed significantly from that in the light-treated unoperated female group (Table 1).

Oestrogens are known to be porphyrinogenic⁵, so we examined the effect of ovariectomy on rats in the light-dark-DDC regime. Again the difference between light and dark on hepatic ALA-S activity was abolished (Table 2b).

As the pineal gland has been implicated in the control of the reproductive system of the rat⁹, we next investigated the effect of pinealectomy. Sham operated controls had part

Table 2 Effect of various treatments on female rat hepatic ALA-S activity

a, Orbitectomy		
Light + DDC:	148.57 $\pm$ 67.89 (12)	} *
Dark + DDC:	150.15 $\pm$ 80.08 (11)	
b, Ovariectomy		
Operated	{ Light + DDC: 80.96 $\pm$ 67.89 (12) } †	} ‡
	{ Dark + DDC: 126.32 $\pm$ 37.59 (8) }	
Sham operated	{ Light + DDC: 279.01 $\pm$ 155.49 (11) } ‡	} §
	{ Dark + DDC: 153.57 $\pm$ 100.89 (10) }	
c, Pinealectomy		
After 7 d	{ Light + DDC: 210.82 $\pm$ 128.98 (12) } §	} ¶
	{ Dark + DDC: 134.37 $\pm$ 60.52 (12) }	
After 100 d	{ Light + DDC: 165.62 $\pm$ 44.06 (8) } ¶	}
	{ Dark + DDC: 153.71 $\pm$ 75.71 (8) }	
Sham operated	{ Light + DDC: 187.53 $\pm$ 74.05 (10) }	}
	{ Dark + DDC: 85.04 $\pm$ 52.32 (9) }	

Values as in Table 1.

* Between light and dark, no significant difference (n.s.); between light-treated orbitectomised and light-treated unoperated female in Table 1, $0.02 > P > 0.01$.

† Between light and dark n.s.; between light-treated ovariectomised and light-treated normal group, $P < 0.001$.

‡ Between light and dark treatment, $0.05 > P > 0.02$.

§ Between light and dark treatments, and between the 7 and 100 d past operative groups, n.s.

¶ Between light and dark treatment, n.s.

|| Between light and dark treatment, $0.005 > P > 0.001$.

of the calvarium and pia mater overlying the pineal gland removed but the gland was left intact and the wound sutured. In one group of pinealectomised rats, the light-dark-DDC regime was started 7 d later, in another group 100 d after operation. (In the latter group, body weight range was 300–400 g). The results in Table 2c show no significant difference between pinealectomised animals, whether kept in the light or the dark, or whether pinealectomy was 7 d or 100 d before the light-dark-DDC regime.

In rats, intact visual pathways are necessary for normal pineal activity and ovarian development^{8,9}, so light may act on haem biosynthesis in the liver via the retina, pineal and gonad. Preliminary results suggest that cycles of 8–12 h light and 16–12 h dark in the light-dark-DDC regime have much the same effect on ALA-S activity as continuous light compared with continuous dark, so that our results may be due to circadian fluctuations in some enzyme or other substance acting in the presence of oestrogen.

Coproporphyrin has been demonstrated in the nervous system of birds¹⁰, where it may play a part in the connection between photoperiodicity and reproductive function.

Continuous darkness or continuous light has been shown¹¹ to alter the activity of two hepatic enzymes in rats, hexobarbital oxidase and *p*-nitroanisole-O-demethylase. We now add evidence that abnormal hepatic metabolism may be under photic influences. The possible influence of environmental lighting on human hepatic porphyria should therefore be borne in mind, although there is so far no evidence for it.

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Biphasic effect of cyclic AMP on an immune response

AGENTS which enhance intracellular adenosine 3', 5'-monophosphate (cyclic AMP) levels by inhibiting cyclic AMP phosphodiesterase activity can increase the number of antibody forming cells (AFC) in humoral immunity both *in vivo*^{1,2} and *in vitro*^{3,4}. Thus, cyclic AMP is a potential

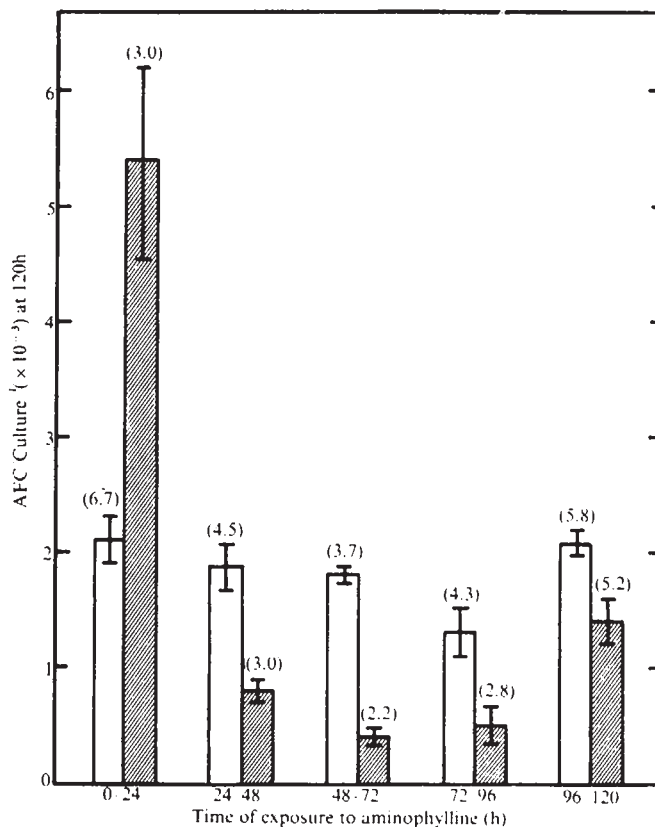


Fig. 1 Effects of aminophylline on the anti-sheep erythrocyte response *in vitro*. Spleen cells from normal CBA/J mice were cultured in Eagle's minimal essential medium containing 10% foetal calf serum, in the presence of 0.01% sheep erythrocytes (SRBC). Each culture initially contained 20×10^6 spleen cells, and was carried out in triplicate in the Diener-Marbrook culture system^{12,13}. At the end of the exposure to aminophylline, the cells were washed by centrifugation, and taken up in fresh medium for further culturing in the presence of antigen. Anti-sheep erythrocyte AFC were determined at 120 h by the method described elsewhere¹⁴. The control cells (no aminophylline) were in each case handled the same way as the experimental cultures. Numbers in parentheses indicate the viable cells, in millions, per culture at 120 h. There was no adjustment of cell numbers after exposure to aminophylline. Shaded columns: cells + medium + 0.01% SRBC + 1 mM aminophylline; unshaded columns: cells + medium + 0.01% SRBC.

mediator of immune induction. But the same agents, at higher than optimal levels, can inhibit the AFC response^{1,3}. Experiments have been done suggesting⁵ that enhanced intracellular cyclic AMP is a mediator of immune paralysis, or tolerance. The apparently contradictory conclusions drawn from these various experiments may be resolved if one considers a common kinetic feature of cyclic AMP responses to various agents, in lymphoid⁶⁻⁸ and in non-lymphoid^{9,10} cells; namely, that the rise in cyclic AMP level is transitory, and the cyclic nucleotide level returns to control values after a few minutes or hours. A similar transient rise in cyclic GMP has been noted in phytohaemagglutinin-stimulated peripheral leukocytes, where this nucleotide seems to mediate the blastogenic response to the mitogen¹¹. Thus, the same agents which might enhance an AFC response if present during the increased cyclic AMP phase, could inhibit the response if present during the subsequent phase of declining intracellular cyclic AMP. To see if this pattern was in fact part of an immune response *in vitro*, we performed experiments with sheep erythrocytes as immunising antigen, and varied the time of exposure of the immunocompetent cells to the agents N⁶, 2'-O-dibutyryl cyclic AMP or aminophylline. When present only during the first 12 or 24 h of exposure to antigen, these

Additives during first incubation (0-12h)	Viability after first incubation	Viable cells on day 4, $\times 10^{-6}$	Anti-SRBC AFC on day 4
SRBC only	98%	3.3	3,940 $\pm$ 270
SRBC + 1mM dibutyl cyclic AMP	73%	4.1	16,990 $\pm$ 770
Medium only	91%	3.4	3,000 $\pm$ 440
1 mM dibutyl cyclic AMP	68%	4.1	3,100 $\pm$ 200

Fig. 2 Kinetic analysis of the effects of cyclic AMP phosphodiesterase inhibitors on the anti-sheep erythrocyte AFC response. Spleen cells were exposed to the agents indicated for the first 12 h in 60 × 15 mm Petri dishes. They were then transferred to the usual Diener-Marbrook culture flasks, and cultured in quadruplicate until the times indicated. Each culture was adjusted to contain 11 × 10⁶ viable cells at the end of the 12 h period. Because of lower viability in treated cultures (Table 1), these represent 30–40% more starting cells than do the controls. The numbers in parentheses indicate the number of viable cells, in millions, per culture at day 5. Vertical bars give the standard errors of the means for the control and dibutylryl cyclic AMP treated cultures (the aminophylline treated values had similar standard errors to the latter) (●) untreated cells, given the same wash and incubation procedures; (△) cells treated with 1 mM dibutylryl cyclic AMP for 12 h first; (▲) cells treated with 1 mM aminophylline for the same period. Antigen was present throughout the culture period, including the first 12 h, in all flasks.

these were smaller than the effects reported by Watson *et al.*, perhaps because of differences in cells (C57BL/6 compared with CBA/J) or culture conditions. At any rate, they were not large enough to obscure our key finding, the antigen dependent stimulatory effect of cyclic AMP phosphodiesterase inhibitors at early times of immune induction. Preliminary experiments in our laboratory show that these agents act in a similar manner on a T cell independent antigen, the polymerised flagellin from *S. adelaide*. Our conclusions agree with those of Watson *et al.* inasmuch as the phase one (commitment) to phase two (proliferation) switch is inhibited by cyclic AMP, but add to them the important observation that an early enhancement of cyclic AMP increases the number of AFC observed at much later times. If the assumption that aminophylline and dibutyl cyclic AMP elevate intracellular cyclic AMP levels is correct, the present work supports a model of primary immune induction in which a temporally limited rise in cyclic AMP is an early positive signal following antigen recognition. Immune paralysis might follow if the cells concerned were unable to reduce the pulse of cyclic AMP to levels consistent with cell cycling.

A preliminary report¹⁹ describes a similar pattern of effects of cyclic AMP enhancing agents in an *in vivo* immune response to sheep erythrocytes. In that system, an early rise in cyclic AMP appears to be followed by its return to normal levels by 1 h.

An inhibitory effect of exogenously added cyclic AMP on the anti-sheep erythrocyte response *in vitro* has been observed by others²⁰. We have not been able to affect the immune response in any way by adding up to 1 mM cyclic AMP itself to cell cultures. It is perhaps not surprising that at least under some conditions this nucleotide is without effect, since the half life of cyclic AMP in rat thymocyte cultures is less than 30 min, and no exogenously added cyclic AMP enters the cells²¹.

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Plasma cell surface antigen on human blood lymphocytes

THERE is considerable evidence that lymphocytes bearing immunoglobulins on their surfaces are present in the peripheral blood of various animal species as well as man¹⁻³. Takahashi *et al.*⁴ indicated that neoplastic, as well as normal mouse plasma cells, contained specific surface alloantigens. These plasma cells do not, however, contain surface immunoglobulin determinants⁵. Harris *et al.*^{6,7} demonstrated that neoplastic plasma cells of the mouse could be used to prepare specific antisera against normal plasma cells. These anti-plasma cell sera (APS) were found to suppress specifically primary and secondary humoral immunity without apparently affecting cellular immunity. Recently, we established a long term culture of human plasma cells⁸ from a localised plasmacytoma tumour of a patient with multiple myeloma. The cultured cells morphologically resembled plasma cells, and they secreted an IgG immunoglobulin antigenically identical to the patient's myeloma protein. Using cells from this source, an anti-human plasma cell serum (HuAPS) has been prepared which recognises a surface antigen on human plasma cells.

Human plasma cells from the long term cell line⁸ were injected into rabbits in the same manner as in the prepara-

Table 1 Effect of APS on mitogenic response of human lymphocytes

<i>In vitro</i> assay	No. studied	c.p.m.	% reduction
Cells alone	8	1,298	
Cells and PHA	9	293,660	
Cells and PHA and APS	6	277,863	5
Cells and PWM	9	175,427	
Cells and PWM and APS	5	97,325	45

tion of rabbit anti-mouse plasma cell sera^{6,7}. The HuAPS was absorbed extensively with lymphoblast cell line RPMI 4098, which was chosen because it has been used for the preparation of anti-human lymphocyte globulin with potent T lymphocyte immunosuppressive activity⁹. Immunoabsorbents were used to purify the HuAPS further. The HuAPS was passed through an immunoabsorbent column prepared by conjugating human immunoglobulins to Sepharose 4B by cyanogen bromide activation.

Ten to twenty millilitres of venous blood was collected from each subject, all of whom were laboratory personnel. Ficoll-Hypaque density centrifugation, as modified from Boyum¹⁰, was used to prepare a relatively pure population of lymphocytes. In some cases, contaminating red cells were lysed by Tris-buffered ammonium chloride.

Human peripheral blood lymphocytes were cultured by the micro method of Hartzman *et al.*¹¹ using 2×10^5 lymphocytes per well in Falcon microtest plates. AB+ serum was used in a 25% concentration with HEPES-buffered RPMI 1640 media. Cells were either incubated

Table 2 Percentage of human blood lymphocytes with membrane antigens

Source of cells	IgG mean/range	IgM mean/range	IgA mean/range	Plasma cell Ag† mean/range
Normal human adults	14.8/6.1-29 (13)*	5.7/2-13 (8)	4.8/1-10 (8)	12 /7-18 (23)
Control <i>in vitro</i> culture	6.3/4-9 (5)	1.5/0-3 (4)	1.8/0-7 (5)	16.9/5-38 (9)
PHA <i>in vitro</i> culture	5.5/4-7 (4)	6.0/4-10 (4)	2.3/0-7 (4)	17.5/12-29 (7)
PWM <i>in vitro</i> culture	14.7/10-20 (5)	2.7/0-8 (5)	2 /0-7 (4)	39 /20-58 (9)

* Figures in parentheses indicate No. of individuals studied.

† Plasma cell surface antigen.

alone, with phytohaemagglutinin (Difco, PHA-P, No. 3110-56, 2 λ per culture); with pokeweed mitogen (Gibco, PWM, No. 536, 2 λ per culture), with PHA plus HuAPS, or with PWM plus HuAPS. The concentration of HuAPS was 2 λ per culture. This was observed to be noncytotoxic. After 4 d of growth, 1 μ Ci of 3 H-thymidine (specific activity 1.9 Ci mmol $^{-1}$) was added to each culture. Quintuplicate cultures from each donor were collected 4-6 h later using glass fibre filter paper precipitation. Filter disks were counted in a Packard Tri-carb liquid scintillation counter. The uptake of 3 H-thymidine was expressed as c.p.m. Table 1 shows that HuAPS had no effect on human peripheral blood lymphocyte response to PHA, a known T-lymphocyte mitogen^{12,13}. HuAPS, however, depressed the PWM response approximately 45%. This was significant for the following reasons. (a) PWM is a mitogen for both T and B lymphocytes. (b) Cells which appear morphologically to be plasma cells form after PWM stimulation. (c) Immunoglobulin synthesis is enhanced by PWM (refs 13, 14). Therefore, it seems likely that HuAPS in the study reported here was blocking a B lymphocyte response.

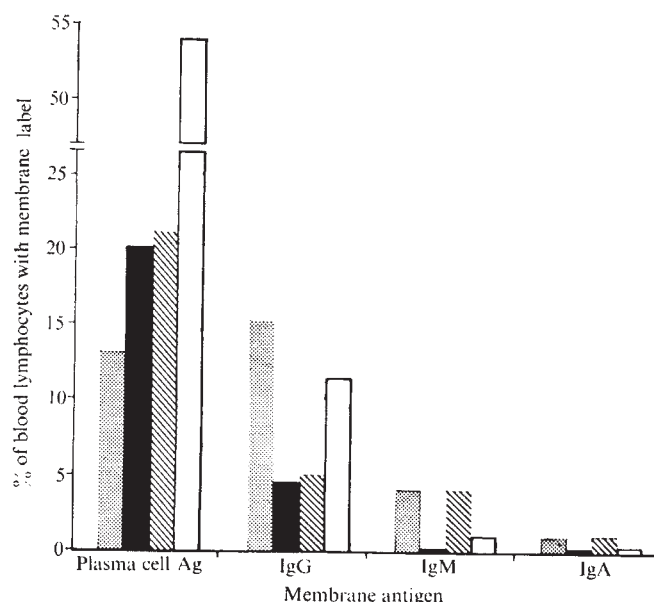


Fig. 1 The membrane staining characteristics of human peripheral blood lymphocytes and cultured lymphocytes from the same individual. Dotted columns, normal peripheral blood lymphocytes; solid columns, control *in vitro* culture; hatched columns, PHA *in vitro* culture; open columns, PWM *in vitro* culture.

Blood lymphocytes with membrane-bound immunoglobulins or with membrane-bound plasma cell antigen were detected by immunofluorescence¹. Cells with membrane-bound immunoglobulin were enumerated by using fluorescein-conjugated goat anti-human IgG, goat anti-human IgM and goat anti-human IgA (all from Cappel Laboratory). Before use, these conjugates were absorbed with cell line RPMI 4098 and with cultured plasma cells. These conju-

gated antisera were then diluted 1:2 and used to stain blood lymphocytes directly. Those used in double-labelling studies with the HuAPS were also absorbed with rabbit sera. For the enumeration of plasma cells, indirect fluorescent staining was used, with either fluorescein or rhodamine-conjugated goat anti-rabbit IgG (Cappel Laboratory). Both conjugated goat anti-rabbit preparations were purified by passage through an immunoabsorbent column conjugated with human serum, and by absorption with human peripheral blood lymphocytes.

To ensure that the HuAPS did not react with membrane-bound immunoglobulins, a blocking experiment was performed. The lymphocytes were incubated with unlabelled goat anti-human immunoglobulins before staining with rabbit anti-human plasma cell serum (HuAPS) and fluorescein-conjugated goat anti-rabbit IgG. In addition, double-labelling studies were performed in which the cells were treated sequentially with HuAPS, rhodamine-conjugated goat anti-rabbit IgG and fluorescein-conjugated goat anti-human IgG, IgM or IgA. Because of a possible peculiarity of the surface binding sites, the order of the procedure was reversed. Fluorescein-conjugated goat anti-human IgG, IgM or IgA was used first, followed by unlabelled HuAPS, and then by a rhodamine-conjugated goat anti-rabbit IgG.

The frequency of blood lymphocytes with membrane-bound immunoglobulins from normal individuals was similar to those reported previously^{2,15} (Table 2). It was also found that the number of lymphocytes with membrane-bound immunoglobulins in PHA or PWM-treated cultures either remained unchanged or decreased. Approximately 12% of peripheral blood lymphocytes had the plasma cell antigen. Ring staining and cap staining patterns were observed for both surface immunoglobulins and the plasma cell antigen. During the mitogenic response of human peripheral blood lymphocytes to PWM, there was an increase in the number of cells exhibiting the plasma cell surface antigen (Table 2). Figure 1 is a representation of an individual's typical membrane-staining distribution for immunoglobulins and plasma cell antigens on lymphocytes which were or were not exposed to mitogens.

The relative frequency of fluorescein-stained cells did not change when the cells were pretreated with unlabelled goat anti-human immunoglobulin. From the double-labelling studies, no cell was found with both the plasma cell surface antigen and the membrane-bound immunoglobulin. Therefore, I concluded that the plasma cell antigen did not cross react immunologically with surface immunoglobulin molecules. The blocking and double-labelling studies also support the concept that the plasma cell antigen is a distinct antigen on the surface of a significant number of peripheral blood lymphocytes; however, it is not known whether the antigen is expressed on nonlymphoid cells.

The observations of Takahashi *et al.*⁴ and Harris *et al.*^{6,7} led to the assumption that neoplastic human plasma cells could have antigens in common with normal human plasma cells. My study indicates that an anti-human plasma cell serum prepared against cultured human neoplastic plasma cells seems to have antibody activity against some normal human blood lymphocytes. (a) Stimulation with PWM, an agent which affects B and T lymphocytes^{13,14} was depressed

to the same extent that rabbit anti-mouse plasma cell sera modified the mouse system (unpublished observations), whereas, stimulation with PHA, a mitogen which affects primarily T lymphocytes^{12,13} was not affected. (b) Immunofluorescent examination showed that a population of blood lymphocytes had the plasma cell antigen, even though these cells do not resemble plasma cells morphologically. This is not surprising, however. Previous studies⁸ demonstrated that cultured plasma cells in spinner culture maintain the morphology of lymphoblasts. When transferred to Falcon tissue culture flasks, they assumed the typical configuration of plasma cells. Thus motion seemed to change the cultured plasma cell from a lymphoid appearance to a typical plasma cell morphology. Circulating plasma cells may not be recognised in multiple myeloma. Most patients examined had 90% or more plasma cells in their bone marrow. It is rare to find morphologically typical plasma cells in their peripheral blood, but metastatic plasma cell lesions are common.

As well as finding circulating blood lymphocytes with a plasma cell antigen, I have demonstrated that cells bearing the plasma cell antigen increased as a result of PWM stimulation. Since the number of immunoglobulin-bearing cells either decrease or remain the same in such cultures, it seems possible that the plasma cell surface antigen could develop as a consequence of stimulation of a certain population of B lymphocytes. This might suggest that the immunoglobulin-bearing cells are precursors of plasma cells. Future studies must examine the relationship between cytoplasmically stained immunoglobulin-containing cells and those cells bearing the plasma cell antigen on their surfaces. Another possibility is that plasma cell precursors migrate from the bone marrow into the peripheral blood. These blood-borne cells are then influenced to undergo further morphological changes and functional differentiation in the microenvironment of the peripheral lymphoid tissue and are consequently recognised as plasma cells.

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Linkage and rearrangement of genes encoding mouse immunoglobulin heavy chains

THE study of the genetic control of immunoglobulin synthesis has been greatly hampered by the difficulty of detecting individual genes encoding variable regions (*V* genes). The distinction by allotypy of several *V*-gene loci in the rabbit is useful, but genetic studies on this species have obvious disadvantages. The discovery in inbred mice of crossreactive idiotypes^{1,2} and of their polymorphic expression³⁻⁶ allows a new approach to the genetic analysis of mouse immunoglobulins. The results presented here suggest that idiotypes may serve to distinguish individual *V*-gene loci within the mouse heavy chain linkage group and to detect recombinations between them.

We have investigated linkage relationships between two mouse antibody idiotypes, termed ARS (ref. 3) and A5A (ref. 4), both of which are linked to the strain A/J *C_H* allotype Ig-1.e (refs 7-9). Idiotypic ARS is associated with antibodies to the *p*-azophenylarsenate hapten, produced in all A/J mice^{3,7} whereas idiotype A5A is associated with antibodies to Group A streptococcal carbohydrate (A-CHO), produced in 94% of A/J mice^{4,8}. It is therefore proposed that strain A/J antibodies to these antigens are at least in part controlled by the *V_H* gene loci *ARS*+ and *A5A*+, respectively. Strain BALB/cJ (Ig-1.a) antibodies to both antigens lack these idiotypes^{7,8}. As was expected from the previously established linkage of each of the idiotypes to the same *C_H* allele^{7,9}, the results confirm that the two idiotypes are specified by linked genes.

We were fortunate to encounter a backcross mouse which was phenotypically suggestive of a recombination between A/J *V_H* genes and BALB/cJ *C_H* genes⁹. This permitted us to investigate whether or not *ARS*+ and *A5A*+ map at the same position within the A/J heavy chain linkage group. The backcross mouse, referred to as BB37, was identified as the only A5A positive mouse among 16 Ig-1.a/a homozygous progeny from an (A/J × BALB/cJ)*F*₁ × BALB/cJ mating⁹. The association of the A5A idiotype with the Ig-1.a allotype, suggesting a new linkage of *A5A*+ with Ig-1.a, has not been observed among more than 100 BALB/cJ mice, and has not been found again among 57 additional backcross segregants tested.

We ascertained the allotype and the presence or absence of two idiotypes in individual mice. Allotypes were determined

Table 1 Linked segregation of *A5A*+, *ARS*+ and Ig-1.e in (A/J × BALB/cJ)*F*₁ × BALB/cJ backcross mice

Mouse, Sex, No.	Ig-1	A5A*	ARS†
1. Litter ♂1	a/a	—	—
♂2	a/e	++	+++
♂3	a/a	—	—
♂4	a/e	+++	+
♂5	a/e	+++	+
♀1	a/e	+	++
♀2	a/e	+++	++
♀3	a/e	+++	+
♀4	a/e	++	+
2. Litter ♂1	a/a	—	—
♂2	a/e	++	++
♂3	a/a	—	—
♂4	a/a	—	—
♂5	a/a	—	—
♀1	a/a	—	—
♀2	a/e	+	++
♀3	a/e	+	++
♀4	a/a	—	—
♀5	a/e	+++	++

* — : <0.2 µg A5A idiotype ml⁻¹.

++ : 40–100 µg idiotype ml⁻¹.

+++ : 100–250 µg idiotype ml⁻¹.

++++ : >250 µg idiotype ml⁻¹.

† — : <50% inhibition by >5 µl of antiserum

++ : >50% inhibition by 0.2 µl of antiserum

+++ : >70% inhibition by 0.2 µl of antiserum

++++ : >90% inhibition by 0.2 µl of antiserum

by agar diffusion analysis using antisera prepared by cross immunisation between strains A/J and BALB/cJ (ref. 10), as previously described⁹. The determination of idiotypes ARS and A5A used indirect radioprecipitin inhibition tests, using rabbit anti-idiotypic antisera for ARS (ref. 3) and guinea pig anti-idiotypic antisera for A5A (ref. 8), respectively. Hyper-immune serum samples were tested for inhibitory capacity in at least three dilutions. Antibodies to A-CHO were determined by a modified Farr assay⁴ and the presence of antibodies to the *p*-azophenylarsonate group was determined by agar diffusion analysis³.

First we had to establish that A/J antibodies to phenylarsonate do not idiotypically crossreact with A/J antibodies to A-CHO and *vice versa*. Sixteen individual anti-phenylarsonate antisera were tested for their capacity to inhibit A5A idiotype binding and 19 individual anti-A-CHO antisera were tested for their capacity to inhibit ARS idiotype binding. Whereas each of these antisera was strongly inhibitory of its corresponding idiotype binding system, less than 10% inhibition was obtained in the heterologous idiotype binding systems, using up to 20 μ l of each undiluted antiserum. This shows the absence of idiotype cross reactivity between strain A/J antibodies to phenylarsonate and to A-CHO, respectively, and enabled us to determine both idiotypes in individual mice which were successively immunised with both antigens.

Table 2 Segregation of A5A+ in BB β 7 $\times$ BALB/cJ progeny and its dissociation from ARS+

Mating, Sex, No.	Ig-1	A5A*	ARS*
BBβ7 $\times$ BALB/cJ†			
1. Litter			
♂1	a/a	—	—
♂2	a/a	+	—
♀1	a/a	—	+/-‡
♀1	a/a	—	—
♀3	a/a	++	—
2. Litter			
♂1	a/a	++	—
♂2	a/a	—	—
♂3	a/a	—	—
♂4	a/a	+	—
♀1	a/a	—	—
♀2	a/a	++	—
♀3	a/a	+++	—
♀4	a/a	—	—
BBβ1 $\times$ BALB/cJ†			
♂1	a/a	—	n.t.
♂2	a/a	—	n.t.
♂3	a/a	—	n.t.
♂4	a/a	—	n.t.
♂5	a/a	—	—
♀1	a/a	—	—
♀2	a/a	—	n.t.
♀3	a/a	—	—
♀4	a/a	—	—
♀5	a/a	—	—

*See footnotes to Table 1.

†The BALB/cJ female was the same in both matings.

‡56% inhibition with 10 μ l of antiserum, doubtful typing.

In a second experiment, a number of (A/J $\times$ BALB/cJ) F_1 $\times$ BALB/cJ backcross mice, which will be referred to as ABB mice, were tested for allotype and for both idiotypes. ABB mice were bred in Cologne from A/J and BALB/cJ mice which were obtained from the Jackson Laboratory, United States. At 8–10 weeks of age the mice were bled and allotyped. Subsequently, the mice received one course of injections with Group A streptococci⁴. The anti-A-CHO antisera resulting from this immunisation were allotyped and then idiotyped in the A5A idiotype binding inhibition assay. After 8–10 weeks, the mice were immunised with KLH-*p*-azophenylarsonate as previously described³. The anti-phenylarsonate antisera resulting from this immunisation were allotyped and then sent to Chicago for idiotyping in the ARS idiotype binding inhibition assay. All experiments were done blind; the source and allotype

Table 3 Expression of ARS+ and A5A+ antibodies in various inbred strains

Strain	Ig-1	ARS	A5A
A/J	<i>e</i>	+	+
A/He*	<i>e</i>	+	+
AL/N	<i>d</i>	+	—
CAL-20†	<i>d</i>	+	—
RF/J	<i>c</i>	—	+

*These mice were immunised for A5A typing by M. Cramer, Basel Institute for Immunology.

†These mice were given to us by Drs M. Potter and E. Mushinsky, N.I.H.

of transferred serum samples were unknown to the investigator performing idiotype analysis.

Table 1 shows the results from allotyping and idiotyping 19 ABB mice. Among three successive allotype determinations on each mouse no discrepancy was observed, so that the allotypes can be taken as a true reflection of the C_H genotype for each mouse. All 11 *Ig-1.a/e* heterozygotes express ARS as well as A5A, whereas all eight *Ig-1.a/a* homozygotes express neither of the two idiotypes. This confirms the linkage between ARS+, A5A+ and *Ig-1.e* in the A/J heavy chain linkage group. The question remains as to whether ARS+ and A5A+ are controlled by a single gene or by different genes.

Therefore, in a third experiment, progeny of a BB β 7 $\times$ BALB/cJ mating were allotyped and idiotyped in the same way as were the ABB mice. We included as a control another litter from the same BALB/cJ female sired by BB β 1, an *Ig-1.a/a* homozygous, but A5A negative (that is non-recombinant) brother of BB β 7 (ref. 9). The results are shown in Table 2. All of the 13 offspring from BB β 7 were *Ig-1.a/a* homozygous, confirming the *Ig-1.a/a* homozygosity of BB β 7. Six of the 13 mice clearly expressed idiotype A5A and thus had inherited the A5A+ - *Ig-1.a* haplotype of BB β 7. Seven of the 13 mice were A5A negative and had inherited the A5A- - *Ig-1.a* haplotype of BB β 7. In contrast, with one doubtful exception, all of the 13 mice were negative for the ARS idiotype, suggesting that the A5A+ - *Ig-1.a* haplotype does not contain the ARS+ gene. The data on the BB β 1 offspring confirm that the A5A+ - *Ig-1.a* haplotype was indeed inherited from BB β 7.

The results are consistent with the view that, in the heterozygous parent of BB β 7, A5A+ was separated by crossover from ARS+ as well as from *Ig-1.e*, and became linked by recombination to *Ig-1.a* of the BALB/cJ chromosome. In this case, ARS+ would map between A5A+ and *Ig-1* and the crossover had occurred between two distinct V_H gene loci. The alternative explanation is a mutation in the BALB/cJ V_H genes from A5A- to A5A+, without ARS- being affected. Such a mutation occurring in one cistron encoding both A5A- and ARS- V_H regions would be unlikely. Therefore, in the case of a mutational event, the data would be indicative of multiple V_H gene loci in strain BALB/cJ.

Though a mutational event is not ruled out, the case for two different germ line V_H gene loci for ARS+ and A5A+ is supported by the association of ARS+ and A5A+ antibodies with several distinct *Ig-1* genotypes (Table 3). Strain AL/N and its *Ig-1* congenic strain CAL-20 express ARS+ but do not express A5A+, whereas strains A/J and A/He express both idiotypes, and strain RF/J expresses A5A+ only¹¹. The separate as well as joint expression of these idiotype markers in different inbred strains, together with their dissociation in the BB β 7 progeny, strongly suggests that A5A and ARS map at different positions in the mouse heavy chain linkage group, and thus represent separate gene loci.

This seems to be the first demonstration of two distinct germ line V_H genes controlling distinct antibody specificities. The data suggest that the establishment of recombinant inbred strains from mice such as BB β 7, and their investigation with multiple idiotype markers, may reveal considerable information on the chromosomal arrangement of antibody genes.

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Interaction of paramyxovirus with erythrocyte membranes modified by concanavalin A

THE envelopes of paramyxoviruses have neuraminidase, haemagglutinating, haemolytic and fusion activities¹⁻⁶. The interrelation of these activities is poorly understood.

Burnet and Lind⁷ studied the interaction of Newcastle Disease virus with several species of mammalian and avian erythrocytes and found most of the cells susceptible to haemagglutination and haemolysis except horse erythrocytes, which agglutinated poorly and resisted haemolysis by the virus. Horse erythrocytes are also not susceptible to haemagglutination by Haemagglutinating Virus of Japan (HVJ: Sendai virus) (refs 8-10 and our own observations). It can be assumed that horse erythrocytes have no specific receptor for the viral haemagglutinin and/or haemolysin¹¹.

Yamakawa¹² reported that horse erythrocytes had no detectable amounts of N-acetyl-neuraminic acid either in the glycolipid fraction or in the residue extracted extensively with organic solvent. Instead, they were found to contain N-glycolyl-neuraminic acid exclusively. It is well known that erythrocytes from which the neuraminic acid residue is removed by the action of the so-called receptor destroying enzyme (neuraminidase) are completely resistant to agglutination and haemolysis by myxo and paramyxoviruses. These facts clearly indicate that the receptor for the viruses contains the neuraminic acid residue. N-glycolyl-neuraminic acid may not have a role as a virus receptor.

The initial interaction of virus with host cell membrane may be that between viral haemagglutinin and the receptor containing N-acetyl-neuraminic acid. It is not known whether this binding of the virus with host membrane is essential for the subsequent haemolysis or fusion. It would be of special interest, therefore, if HVJ proved to have haemolytic activity when the virus was artificially adsorbed on horse erythrocytes. In this report, we shall show that horse erythrocytes treated with concanavalin A (con A) are able to interact with HVJ and to be haemolysed.

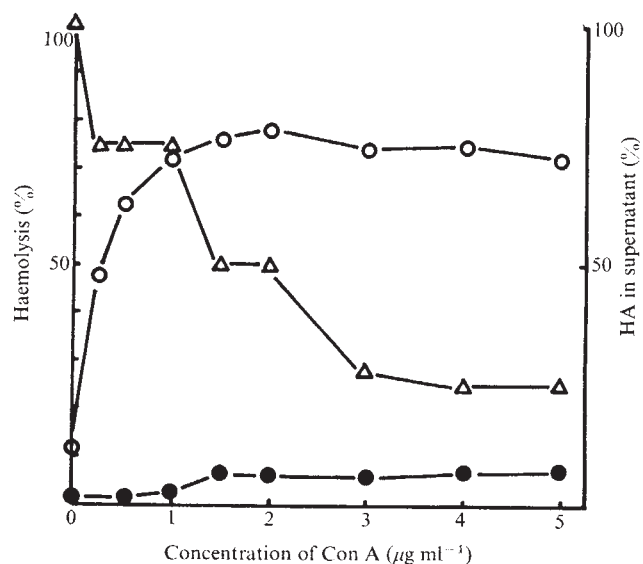


Fig. 1 Adsorption (Δ) and haemolytic activity (O) of HVJ to horse erythrocytes treated by various concentrations of con A. Aliquots of con A solution were mixed with equal volumes of 2% horse erythrocyte suspension in phosphate-buffered saline (pH 7.2). The concentration of con A added was expressed as final concentration. After the treatment with con A at 0° C for 4 h, whole mixtures were directly used without washing, since non-reacting free con A was not detected at the doses used through the present experiment. For the adsorption experiment, 0.1 ml of the virus suspension (4,000 HA per 0.5 ml) was mixed with 0.9 ml of intact and con A-treated horse erythrocytes. After incubating for 2 h at room temperature, HA titre remaining in the supernatant of centrifugation (1,500g, 5 min) was determined. For the haemolysis experiment, 0.5 ml of the same virus suspension (10,000 HA per 0.5 ml) was mixed with 0.5 ml of the same preparation of con A-treated cells as used in the adsorption experiment. After incubation of the mixtures for 2 h at 36° C, the optimum density of clear supernatant obtained by centrifugation (1,500g, 5 min followed by 80,000g, 15 min) was determined photometrically (at 541 nm). Control lysis of con A-treated horse erythrocytes without HVJ was also determined (—●—). Activities were expressed as a percentage of original HA titre or complete haemolysis obtained by hypotonic treatment.

HVJ was highly purified by differential centrifugation, sucrose density gradient centrifugation and finally by gel filtration through a Sepharose 2B column. Fractions in void volume were collected. The pooled fractions were finally concentrated in a reduced volume of phosphate-buffered saline by pelleting at 60,000g for 30 min. The viral sample was exposed to freezing-thawing two or three times to activate the haemolytic activity¹³. The treatment of horse erythrocytes with con A is described in Fig. 1. Horse erythrocytes were purchased as stored blood and used within 3 weeks. The haemagglutinating titre (HA) was routinely determined with chicken erythrocytes. The degree of adsorption of HVJ on con A-treated horse erythrocytes was estimated by titration of the HA remaining in the supernatant after the incubation mixture was centrifuged at low speed. An aliquot of the suspension of con A-treated horse erythrocyte (0.9 ml) was incubated with 0.1 ml of HVJ (4,000 HA per 0.5 ml) for 2 h at room temperature. Haemolytic activity of the virus was assayed as follows: an adequate dilution of HVJ and the same volume of con A-treated horse erythrocytes were mixed and incubated at 36° C for 2 h. After the incubation, nonhaemolysed cells were removed by sedimentation at 1,500g for 5 min. The supernatant thus obtained was further centrifuged at 80,000g for 15 min to remove viral particles. Haemolysis was measured by reading optical density at 541 nm of the final supernatant. For the inhibition experiment of virus-induced haemolysis by sugars, the mixture containing con A-treated horse erythrocytes

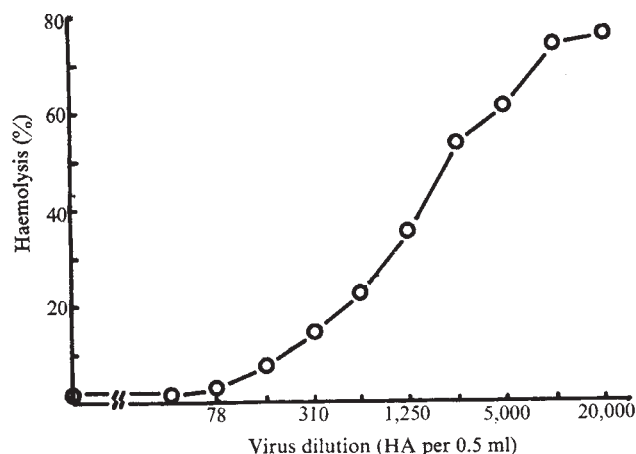


Fig. 2 Haemolysis of con A-treated horse erythrocytes by varying titres of HVJ. Viruses (32,000 HA per 0.5 ml) were diluted with the phosphate-buffered saline by twofold serial dilution of 0.5 ml. Horse erythrocytes were treated with con A at the final concentration of $1 \mu\text{g ml}^{-1}$. Suspensions of con A-treated horse erythrocytes (0.5 ml) were added to each dilution of the virus and haemolysis was assayed by the same procedure as in Fig. 1.

and a monosaccharide was preincubated at 4°C for 30 min. Adequate dilutions of HVJ were added to the mixture and further incubated at 36°C for 2 h. The haemolysis was assayed as described above. A plasma membrane fraction was prepared from intact and con A-treated horse erythrocytes by hypotonic haemolysis. The membrane pellets finally obtained were resuspended in a small volume of phosphate buffered-saline. For the electron microscopic observation, samples were negatively stained with phosphotungstate ($\text{pH } 6.0$).

The adsorption of HVJ on con A-treated horse erythrocytes and the subsequent haemolysis are shown in Fig. 1.

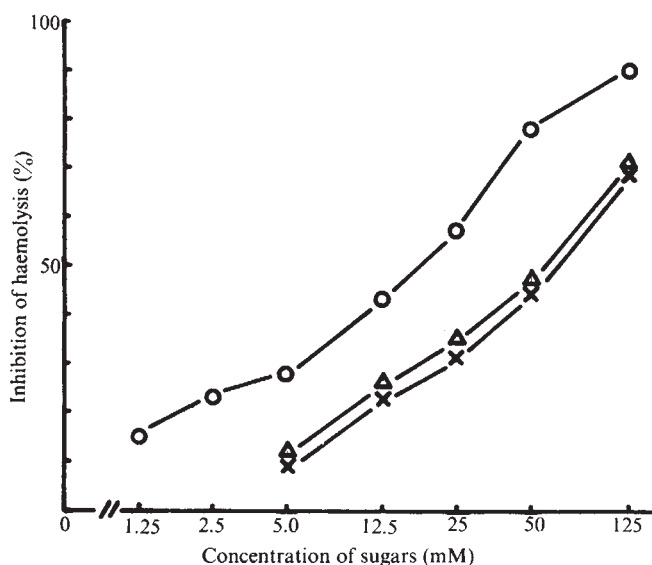


Fig. 3 Inhibitory effect of monosaccharides on haemolysis of con A-treated horse erythrocytes by HVJ. Horse erythrocytes were treated with con A at the final concentration of $1 \mu\text{g ml}^{-1}$ as described in the legend to Fig. 1. Con A-treated cells (0.5 ml) were mixed with 0.25 ml of solutions containing various amounts of (○), α -methyl-D-mannoside; (△), α -methyl-D-glucoside; (×), D-mannose. After incubating the mixtures at 4°C for 30 min, 0.25 ml of HVJ suspension (10,000 HA per 0.5 ml) was added. Haemolysis was assayed after incubating 2 h at 36°C . The degree of inhibition was expressed as a percentage of the haemolysis without sugars.

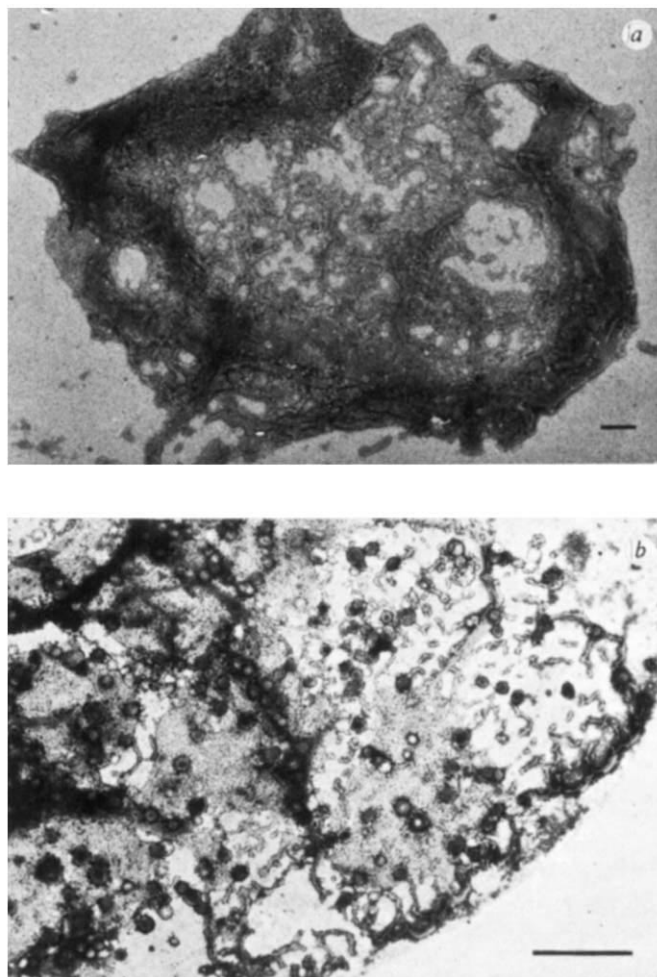


Fig. 4 Electron microscopic observation of adsorption of HVJ on plasma membrane derived from intact and con A-treated horse erythrocytes. Con A-treated horse erythrocytes (final concentration of con A: $5 \mu\text{g ml}^{-1}$) were prepared as described in the legend to Fig. 1. Plasma membranes were prepared by the following procedure. Ten millilitres of intact and con A-treated horse cells were diluted in 30 ml of distilled water and sedimented at 1,500g for 5 min. The pellets obtained were washed three times with phosphate-buffered saline and were resuspended in 0.1 ml of phosphate-buffered saline. The suspension was mixed with 0.1 ml of HVJ (5,000 HA per 0.5 ml) and the mixture was incubated for 30 min at room temperature. After washing three times with phosphate-buffered saline to remove non-adsorbed virus particles, the samples were stained with phosphotungstate ($\text{pH } 6.0$). a, Plasma membrane from an intact horse cell; b, a con A-treated horse cell. Scale: 1,000 nm.

Intact horse erythrocytes showed negligible interaction with HVJ and were not susceptible to viral haemolysis. With the increase in con A concentration, the HA titre remaining in the supernatant decreased, which indicated that more viruses were adsorbed on the cells. The susceptibility of cells to haemolysis increased in parallel with the increase of adsorption of the virus on the con A-treated cells. The degree of haemolysis reached a plateau at $1 \mu\text{g ml}^{-1}$ con A although higher concentrations of con A caused more HVJ to adsorb on to the cells. The haemolysis was also shown to be dependent on virus doses. The maximum haemolysis was obtained at a titre of 10,000 HA per 0.5 ml (Fig. 2). The control haemolysis of con A-treated horse erythrocytes was almost negligible without HVJ even at the highest concentration of con A (Fig. 1). These results suggest that the haemolysis may be due to the direct action of the HVJ virion on the erythrocyte membrane. Influenza viruses which had no haemolytic activity showed almost the same ability to

bind to con A-treated horse erythrocytes as HVJ, although the cells did not reveal detectable haemolysis. This fact may exclude the possibility that con A treatment makes the cells fragile and enhances nonspecific lysis due to the attachment of viral particles.

The effect of con A on horse erythrocytes described above was shown to be influenced by typical inhibitors of the plant agglutinin (Fig. 3). α -Methyl-D-mannoside showed the most potent inhibitory activity against the virus-induced haemolysis, while α -methyl-D-glucose and D-mannose showed much weaker inhibitory activity. It seems that the inhibition of haemolysis by a monosaccharide results from blocking the adsorption step of virus on to blood cells, since the degree of adsorption of the viruses was affected by those inhibitors (not shown in the figure). The pattern of inhibition by sugars shown here is almost the same as those reported for the aggregation of dextran by con A¹⁴. The reversibility of con A action on the blood cell membranes was also confirmed.

The adsorption of HVJ on con A-treated horse erythrocytes was examined under the electron microscope (Fig. 4). Plasma membranes of con A-treated horse cells could adsorb the viruses on the cell surface whereas those of the intact cells could not. The HVJ virion, once it had interacted, could not be removed even by washing extensively by phosphate-buffered saline. Haemolysis of con A-treated horse erythrocytes by the virus showed a similar characteristic to the haemolysis observed in the system of chicken erythrocytes and HVJ (manuscript in preparation).

The mechanism of the interaction between con A-treated horse erythrocytes and HVJ still remains to be clarified. HVJ may be bound to con A-treated horse erythrocytes through a con A bridge, since con A has polyvalent binding sites in its molecule¹⁵ and virions of HVJ have receptors for con A¹⁶. Alternatively, membranes of horse erythrocytes may acquire some new binding sites for the virus or just reveal buried receptors by combining with con A. This is plausible since con A modifies several membrane functions and structures¹⁷⁻¹⁹. Recent studies in which HVJ was fractionated to subcomponents suggested that the viral haemolysin might be on different molecules from the viral haemagglutinin and neuraminidase. These studies failed, however, to show whether the interaction of the virus with host membranes through the receptor for the haemagglutinin and/or neuraminidase is essential for the expression of haemolysis²⁰.

Our results suggest that haemolysis induced by HVJ does not require the binding of viruses to the specific receptors such as sites for haemagglutinin or neuraminidase on cell surface. Even when HVJ was adsorbed on erythrocytes by a different mechanism the cells were haemolysed

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Replication of *Escherichia coli* requires DNA polymerase I

For many years DNA polymerase I was the only known *E. coli* enzyme with the ability to synthesise DNA. It was, therefore, assumed by many to be the enzyme required for DNA replication. Doubts concerning this assumption were taken seriously only when DeLucia and Cairns¹ isolated a viable *E. coli* mutant with less than 1% of the normal polymerase I activity in *in vitro* tests. The conjecture that polymerase I might not be involved at all in replication but only functions in DNA repair processes was soon superseded by the observation that polymerase I is involved in the joining of Okazaki pieces^{2,3}. But the question whether DNA polymerase I is essential for replication or whether it is dispensable enzyme remained open. To investigate whether DNA polymerase I is essential for replication, we attempted the isolation of conditional lethal mutations in the gene coding for polymerase I.

Among many DNA polymerase I mutants of *E. coli* we have isolated one (BT 4113) which is conditional lethal at elevated temperature because of a temperature-sensitive DNA polymerase I. A bacterial culture was mutagenised by N-methyl-nitrosoguanidine and screened for *pol A* mutants using a new replica plating procedure in which microcolonies growing on membranes are tested after lysis *in situ* for their ability to synthesise DNA or to degrade DNA exonucleolytically. This method, which was originally developed for the large-scale isolation of *dna* temperature sensitive mutants will be described in detail elsewhere. Approximately

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Table 1 Temperature dependence of polymerising and 5'-exonuclease activity

Strain	Polymerising activity (pmol TTP incorporated μl^{-1})		Assay for 5'-Exonuclease activity (pmol ^{32}P released)	
	30° C	45° C	30° C	45° C
W 3110	44	140	0.20	0.33
BT 4109	18.3	1.79	0.27	0.31
BT 4113	9.3	0.72	0.16	0.05

DNA polymerase I was partially purified and assayed as described by Lehman and Chien⁴. Sucrose-gradient fractions were prepared and assayed for both polymerase and 5'-exonuclease activity. The polymerising activity was determined at pH 7.5 using calf thymus DNA as a template. The incubation was preceded by a 15-min preincubation under identical conditions. The 5'-exonuclease activity was determined by degradation of 5'- ^{32}P -dTdA over 5 min as described by Lehman and Chien⁴. The activity measured is sensitive to DNA polymerase I antiserum (data not presented).

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40,000 lysed colonies from this culture could be conveniently tested for their polymerase I activity at elevated temperature. Those lysed colonies unable either to synthesise DNA at 45° C in the presence of N-ethylmaleimide (NEM) or to degrade DNA exonucleolytically in the presence of NEM at 45° C were detected by autoradiography and the corresponding colonies were isolated from the replica. NEM inhibits many enzymes including DNA polymerase II and III but not polymerase I.

Among 40,000 bacterial colonies of *E. coli* W 3110 (ref. 1) we found 12 mutants with measurable defects in polymerase I activity, some having as little as 1% of wild-type polymerase activity. As expected¹, the mutants were sensitive to methylmethane-sulphonate. Some mutants had a temperature-sensitive DNA polymerase. The temperature dependence of polymerising and 5'-exonuclease activity for two strains is shown in Table 1. The polymerising activity of both strains was temperature sensitive. The 5' exonuclease activity seemed normal in BT 4109 and was temperature sensitive in BT 4113. When exposed to high temperature, bacteria of strain BT 4113 formed snakes and failed to form colonies. BT 4113 is the only conditional lethal mutant we have found.

DNA polymerase I is known to be involved in sealing of Okazaki pieces^{2,3}. According to sedimentation analysis of newly synthesised DNA the mutants tested varied in their ability to join Okazaki pieces, some (for example BT 4004) being almost as efficient as wild type, some (for example BT 4100 being grossly defective in sealing and some (for example BT 4113) being grossly defective in sealing only at elevated temperatures. In the latter strain there was almost no joining observed at 45° C even after 15 min, whereas joining at low temperature seemed nearly normal.

Transduction experiments with strain BT 4113 showed that both the conditional lethality and the temperature sensitivity in sealing are co-transducible with the *met E* marker at high frequency (about 10%). The transduction has been performed in both directions; BT 4113 has been transduced by P1 to become temperature resistant and *met E*⁻ and the *met E*⁻ strain JW 149 has been transduced to become *met E*⁺ and temperature sensitive.

We have not yet found a mutant to be temperature sensitive in the polymerising part only, and to be conditional lethal because of that mutation. The isolation and characterisation of BT 4113 and of a similar conditional lethal *pol A* strain by Conrad and Lehman⁵ show that *E. coli* bacteria require the 5'-exonucleolytic activity of DNA polymerase I for replication and that this function of polymerase I cannot be carried out by other enzymes in the cell.

The enzyme assays were performed in Professor I. R. Lehman's laboratory. We are grateful to him and his group for their hospitality and patience, and for communicating their results to us before publication. This research was supported by the Max-Planck-Gesellschaft in Germany and by grants from the National Cancer Institute and from the National Science Foundation.

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Affinity labelling the acceptor site of the peptidyl transferase centre of the *Escherichia coli* ribosome

We have identified two 50S proteins, L2 and L26-L27, in the peptidyl (P) site of the peptidyl transferase centre of *E. coli* ribosomes¹. BrAc-³H-Phe-tRNA^{Phe}, a peptidyl-tRNA affinity analogue, was shown to bind specifically to the P site and covalently react with only L2 and L26-L27. The ability of the same molecules of ribosome-bound BrAcPhe-tRNA^{Phe} to participate in dipeptide formation and covalent attachment to ribosomal proteins was the most compelling evidence for functional P site binding.

De Groot *et al.*² showed how peptidyl-tRNAs can be directed to either the P site or the aminoacyl (A) site. For example, purified Gly₃Phe-tRNA^{Phe} binds to the P site of 70S, salt-washed *E. coli* ribosomes in a cell-free system using poly (U) and either 10 mM or 30 mM Mg²⁺. This binding was not inhibited by tetracycline. The bound peptidyl-tRNA was very reactive towards puromycin. In contrast, when excess deacylated tRNA^{Phe} was added together with the peptidyl-tRNA, the distribution between the two sites was different. The greater part of the peptidyl-tRNA was now bound to the A site. This binding was inhibited by tetracycline, and puromycin reactivity of the bound peptidyl-tRNA was almost completely lost. It could be restored by adding translocation factor EF-G and GTP. Apparently, the

Table 1 Binding of BrAc-³H-Phe-tRNA

(a) Binding of BrAc-³H-Phe-tRNA to *E. coli* ribosomes (fmol)

Additions	Salt-washed ribosomes		Non-washed ribosomes	
	10 mM Mg ²⁺	30 mM Mg ²⁺	10 mM Mg ²⁺	30 mM Mg ²⁺
None	74	132	142	141
Tetracycline	123	121	134	133
400 pmol tRNA	47	113	98	124
400 pmol tRNA and tetracycline	34	73	45	91

(b) Puromycin reaction after binding of BrAc-³H-Phe-tRNA (fmol released)

Additions	Salt-washed ribosomes		Non-washed ribosomes	
	10 mM Mg ²⁺	30 mM Mg ²⁺	10 mM Mg ²⁺	30 mM Mg ²⁺
None	88	76	100	98
400 pmol tRNA	16	26	38	23
400 pmol tRNA + EF-G + GTP	—	91	65	98

tRNA_{*E. coli*} enriched in phenylalanine acceptor activity was prepared by one fractionation of crude *E. coli* tRNA on a BD-cellulose column. This tRNA could accept about 50 pmol per A₂₆₀ of ³H-phenylalanine 60 Ci mmol⁻¹ and served also as uncharged tRNA in the specified reactions. *E. coli* ribosomes and BrAcPhe-tRNA were prepared as described previously¹⁴. Salt-washed ribosomes were prepared according to Ertel *et al.*¹⁵ Binding assays were performed by the Millipore filter technique of Nirenberg and Leder¹⁶. A 20 µl binding reaction mixture contained 50 mM Tris-HCl (pH 7.6), 50 mM NH₄Cl, 3.0 A₂₆₀ of ribosomes, 20 µg of poly (U) plus magnesium acetate as indicated. Deacylated tRNA and tetracycline (0.5 mM) were added at 0° C before the addition of 240 fmol (11,000 c.p.m.) BrAc-³H-Phe-tRNA. The mixture was incubated for 30 min at 37° C. Puromycin reaction was determined as described by Leder and Bursztyn¹⁷. Where indicated EF-G (12 µg) and GTP (0.5 mM) were added after the initial incubation. The puromycin reaction in this case was measured after an additional 10 min incubation.

Table 2 Incorporation of ^3H -Phe into ribosomal proteins

	1.D	Run 1 2.D	Run 2 1.D
P site 10 mM Mg^{2+}			
Mg protein applied to gel	0.54	1.80	0.84
c.p.m. ^3H in protein L2	350 (0.076)	1,050 (0.073)	160 (0.27)
L16	75 (0.016)	246 (0.017)	180 (0.030)
L26-L27	2,900 (0.630)	9,840 (0.686)	1,130 (0.189)
P site 30 mM Mg^{2+}			
Mg protein applied to gel	—	—	1.20
c.p.m. ^3H in protein L2	—	—	60 (0.005)
L16	—	—	90 (0.007)
L26-L27	—	—	770 (0.064)
A site			
Mg protein applied to gel	0.45	1.50	0.98
c.p.m. ^3H in protein L2	130 (0.041)	420 (0.039)	170 (0.028)
L16	465 (0.145)	1,375 (0.129)	450 (0.075)
L26-L27	830 (0.260)	5,200 (0.488)	480 (0.080)

The amount of radioactivity found associated with certain ribosomal proteins was determined from the analysis of one-dimensional and two-dimensional polyacrylamide gels as described previously^{1,14}. After each ^3H c.p.m., in parentheses, is shown the corrected covalent c.p.m. ^3H incorporated per equivalent of tRNA bound per mg of protein analysed on the gel. In run 1, P site conditions actually represent 88% P site, A site represent 75% A site as determined by puromycin reactivity. In run 2, the corresponding figures are 71% P site and 75% A site.

deacylated tRNA^{Phe} could compete effectively with the peptidyl-tRNA for binding to the P site, but much less so for binding to the A site. This effect was much more pronounced at 30 mM Mg^{2+} . Watanabe and Tanaka reached similar conclusions³. AcPhe-tRNA could be directed specifically to the A site at 15 mM Mg^{2+} if the ribosomes had been preincubated with excess unfractionated deacylated tRNA.

We have used these methods to direct BrAc- ^3H -Phe-tRNA to either the P site or A site by using tRNA^{Phe} *E. coli*, and by varying the Mg^{2+} concentration. Table 1 shows that BrAc- ^3H -Phe-tRNA behaves similarly in every way to Gly₃-Phe-tRNA and AcPhe-tRNA in a poly(U)-directed cell-free system. This is true for saltwashed and unwashed ribosome preparations. BrAc- ^3H -Phe-tRNA in 10 mM Mg^{2+} and the absence of deacylated tRNA, bound almost exclusively to the P site. It was not inhibited by tetracycline. Puromycin reactivity of the bound tRNA was generally 70% or higher. In the presence of deacylated tRNA and 30 mM Mg^{2+} , however, binding seemed to be mostly to the A site. This binding was inhibited by tetracycline. Puromycin reactivity was reduced by a factor of 3 to 4. It could be restored fully by adding EF-G and GTP.

Covalent labelling reactions were done under similar conditions to those used previously¹. Unwashed ribosomes were used to facilitate comparison with earlier results and because their overall binding was generally higher than saltwashed ribosomes. Two different systems were chosen. (1) P site binding in the presence of 10 mM or 30 mM Mg^{2+} and the absence of deacylated tRNA. (2) A site binding in the presence of 30 mM Mg^{2+} and deacylated tRNA.

Subunit separation and stripping of ribosomal proteins were done as described previously¹. Most of the covalent ^3H -Phe incorporation occurred with 50S proteins. The small extent of reaction with the 30S particles will be discussed elsewhere. One-dimensional and two-dimensional acrylamide gel electrophoreses were performed to identify as unambiguously as possible any covalently labelled 50S proteins. Typical gel patterns are given in Figs 1 and 2.

Only three 50S proteins contained significant covalently incorporated ^3H -Phe. These were L2, L26-L27 and L16. Table 2 shows that the pattern of radioactive incorporation into the three proteins was markedly affected by the conditions used. The good agreement between parallel one-dimensional and two-dimensional analyses on the same sample encourages quantitative comparisons. The P site

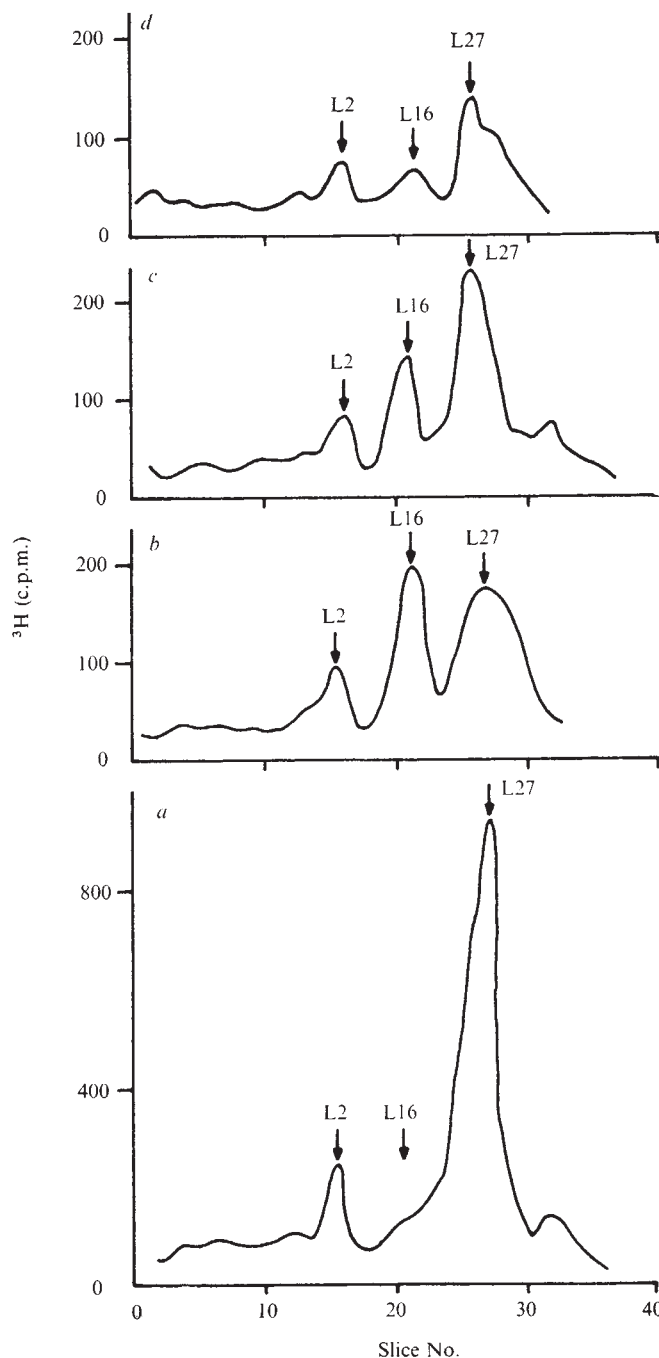


Fig. 1 BrAc- ^3H -Phe-tRNA-reacted 70S ribosomes were separated into subunits and the ribosomal proteins were analysed by one-dimensional gel electrophoresis as described earlier¹⁴. Labelling patterns from one-dimensional gels are shown for 50S proteins from: (a) P site reaction mixture; (b) A site reaction mixture; (c) A site reaction mixture in the presence of 0.5 mM tetracycline; (d) A site reaction mixture in the presence of 1 mM puromycin. Total protein put on the gel was 0.54 mg in (a), 0.45 in (b), 0.48 in (c), and 0.30 mg in (d). Labelling reactions contained 20 mg of ribosomes with 24 pmol (1.1×10^6 c.p.m.) of BrAc- ^3H -Phe-tRNA and 2 mg of poly (U) in the presence of 10 mM Mg^{2+} for P site binding, and 30 mM Mg^{2+} and 1 mg of uncharged tRNA for A site binding. The concentrations of Tris buffer and NH_4Cl were the same as given in Table 1. After incubation for 30 min at 37° C, 20 μl aliquots were taken and checked for binding and puromycin reactivity in the presence and absence of EF-G and GTP. Where indicated either tetracycline (0.5 mM) or puromycin (1 mM) were added to the A site reaction mixtures before the addition of BrAc- ^3H -Phe-tRNA. It was found that 8,520 c.p.m. of BrAc- ^3H -Phe-tRNA were bound to a sample of 0.2 mg ribosomes from the P site reaction mixture as measured by Millipore binding assay¹⁶. 7,450 c.p.m. (88%) of this sample could react with puromycin. The binding to an equivalent amount of ribosomes from the A site reaction mixture was 7,130 c.p.m. of which only 1,760 c.p.m. (25%) were puromycin reactive.

results are quite comparable to previous results¹, except that L2 is less reactive with this particular ribosome preparation. Similar variations have been seen before. For the same preparation of ribosomes and BrAcPhe-tRNA, however, the ratio of L2:L26-L27 in a P site experiment is very reproducible. The A site conditions with the same preparation gave a striking increase in reaction with protein L16 and a decrease in reaction with L2 and L26-L27. The L16 reaction is not simply due to the presence of 30 mM Mg²⁺ as shown in Table 2. In the absence of deacylated tRNA, even with 30 mM Mg²⁺, the binding of BrAcPhe-tRNA is mostly to the P site (Table 1). Only when excess deacylated tRNA is added does BrAcPhe-tRNA move to the A site with a concomitant rise in the L16 reaction.

One reason why the pattern of BrAcPhe-tRNA reactivity shown in Table 2 is complex is that P site and A site conditions still lead to binding to both sites. If puromycin reactivity is used as a measure of true P site binding, P site conditions still allow 12–29% A site binding while A site conditions allow 25% P site binding. These cross-contaminating binding modes can be corrected for by the appropriate simple algebraic manipulation. This permits calculation of the relative efficiencies of reaction of a pure

inhibition of the binding of the aminoacyl end of BrAcPhe-tRNA to the A site would explain the diminished L16 reaction.

Our assignment of L16 as an A site protein may clarify other recent affinity labelling studies. Several lines of evidence^{6–9} indicate that chloramphenicol and puromycin compete for the same binding site on the 50S particle, the A site. Sonnenberg *et al.*¹⁰ observed a covalent reaction of the chloramphenicol analogue, bromamphenicol, with two proteins, L2 and L27. Pongs *et al.*¹¹ found that another analogue, moniodoamphenicol, reacted covalently with a single 50S protein, L16. Reconstitution experiments demonstrated that L16 was required for chloramphenicol binding¹². One explanation for this discrepancy is the known occurrence of two ribosomal binding sites for chloramphenicol¹³. The iodoamphenicol results^{11,12}, however, are consistent with our identification of L16 as exclusively an A site protein. L2 L26-L27 can react with BrAc-³H-Phe-tRNA bound at both the A and P sites. This is consistent with the bromamphenicol results¹⁰. It appears that proteins L16, L2, L26-L27 are all located at the peptidyl transferase centre and are probably quite close to each other.

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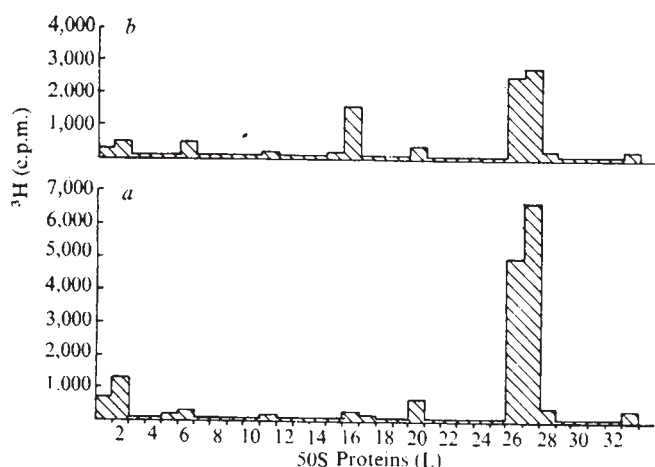


Fig. 2 50S protein samples from reaction mixtures (a) and (b) (see Fig. 1), were analysed by two-dimensional gel electrophoresis as described previously¹.

A or P site bound species with each of the three reactive 50S proteins. Such calculations show that virtually all the small L16 reactivity seen under P site conditions can be accounted for by contaminating A site-bound material. Therefore, L16 is definitely an A site protein. It is vastly more reactive when BrAcPhe-tRNA is bound in this site than in the P site.

L26-L27 and L2 appear to be P site proteins. The reactive sites of L26-L27 and L2 may be reached from both A and P site bound BrAcPhe-tRNA, but the higher efficiency from the latter suggests that they are much closer to the P site or the reaction is sterically more favourable.

Figure 1 shows the effect of 0.5 mM tetracycline and 1 mM puromycin on the pattern of covalent labelling under A site conditions. Tetracycline, a known inhibitor of A site binding diminished the reaction of L16 by a factor of 2. A much smaller effect was seen with L2 and L26-L27. Puromycin which releases any P site-bound peptidyl-tRNA reduced the reaction with L16, L26-L27 and L2. The marked reduction of L16 reaction needs to be explained since 1 mM puromycin is not likely to release any A site-bound peptidyl-tRNA. Lessard and Pestka⁵ have shown that puromycin competition appreciably reduced the binding of aminoacyl oligonucleotides to ribosomes. A similar

Identification of a population of mouse leukocytes using wheat germ agglutinin

A GROUP of proteins occurring commonly in plant extracts have the property of agglutinating blood cells by intercellular linkage of surface carbohydrate groups^{1,2}. These proteins, usually called lectins, are often able to distinguish cell types by preferentially agglutinating cells which express a particular membrane glycoprotein³. We report here that one lectin, wheat germ agglutinin (WGA)⁴, is able to distinguish a group of mouse leukocytes carrying neither immunoglobulin nor T-cell surface markers.

Cells teased from spleens of normal BALB/c mice were washed with Eagle's minimum essential medium containing 10% foetal bovine serum. Suspensions containing 10^7 cells ml^{-1} were treated with increasing concentrations of fluorescein-conjugated WGA and the number of labelled cells counted immediately using a Leitz Ortholux ultraviolet microscope with incident illumination. The results are shown in Fig 1. A consistent proportion of spleen cells was found to be strongly labelled at WGA concentrations of between 20 and $100 \mu\text{g ml}^{-1}$. Staining was eliminated either by previous addition of 0.025 M N-acetyl glucosamine (NAG) to the cell suspension, or by subsequent washing of the cells with Eagle's medium containing 0.05 M NAG. In contrast, washing with 0.1 M NAG had no effect upon spleen cells previously labelled with another fluorescein-conjugated lectin, concanavalin A.

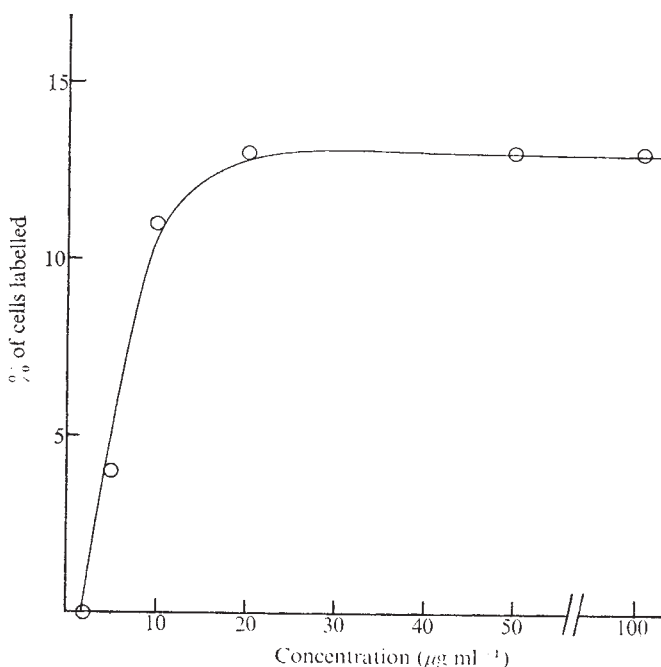


Fig. 1 Proportion of mouse spleen cells labelled with increasing concentrations of fluorescein-conjugated WGA.

Table 1 shows the proportions of cells from other mouse lymphatic tissues having receptors for WGA.

The morphology of this cell population was investigated by electron microscopy. Mouse spleen cells were incubated for 5 min at 4°C with a $250 \mu\text{g ml}^{-1}$ concentration of WGA/horseradish peroxidase conjugate⁵ in phosphate buffered saline. The cells were then washed, fixed and treated with diaminobenzidine and osmium tetroxide before embedding and sectioning. Cells with heavy surface labelling were characterised as monocytes and polymorphonuclears, many with peroxidase-positive cytoplasmic granules. Lymphocytes and plasma cells showed very weak or no staining. Further details of these experiments will be published elsewhere.

To see whether cells with abundant WGA receptors carried immunoglobulin or T-cell markers the following experiment was performed. Aliquots of 2×10^6 cells were treated with either 100 μl of a 1:4 dilution of rabbit anti-mouse immunoglobulin serum or with 100 μl of rabbit anti-mouse brain serum, sequentially absorbed with erythrocytes, liver and B-spleen (spleen from irradiated animals repopulated with bone marrow cells) to render it specific for T lymphocytes⁶. All cells were subsequently

Table 1 Percentage of leukocytes in mouse tissues labelled with WGA ($50 \mu\text{g ml}^{-1}$)

Spleen	Lymph node	Thymus	Blood	Bone marrow
12	<0.5	<0.5	7	72

stained with rhodamine B-conjugated goat anti-rabbit immunoglobulin serum. The cells were then washed thrice and labelled with 100 μl of a $50 \mu\text{g ml}^{-1}$ solution of fluorescein-conjugated WGA. Of the 12% of cells in each sample labelled with wheat germ agglutinin, none showed rhodamine fluorescence indicative of either immunoglobulin or T-cell surface markers. Conversely, T cells were not stained with WGA. Although WGA did not stain immunoglobulin-bearing cells under these conditions, at lectin concentrations greater than $100 \mu\text{g ml}^{-1}$, both immunoglobulin-bearing cells and erythrocytes showed weak fluorescence.

This is consistent with the findings of Schnebli and Dukor⁷ that mouse B cells are agglutinated more strongly than cortisone-resistant thymocytes at low concentrations of WGA. Wheat germ agglutinin has also been shown to agglutinate certain malignant cells⁸. Our results show that mouse leukocytes in lymphoid organs and blood which have abundant binding sites for WGA are essentially of myeloid morphology and do not carry the surface markers of B or T lymphocytes.

We thank Dr A. K. Allen for his gift of purified WGA, Dr C. Maino for a number of other lectins used in this study and Miss M. Gyöngyössy for rabbit anti-mouse brain serum. This work was supported by the Medical Research Council.

Note added in proof: In contrast with resting mouse lymphocytes, lymph node cells transformed with phytohaemagglutinin or concanavalin A were found to bind WGA to a moderate degree.

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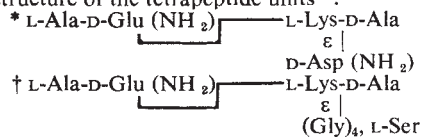
Adjuvant activity in delayed hypersensitivity of the peptidic part of bacterial peptidoglycans

It is generally accepted that the presence of killed mycobacteria in complete Freund's adjuvant is necessary to induce a typical delayed hypersensitivity to soluble antigens¹ and attempts have been made to isolate the active component. The preparations obtained initially were of a peptidogly-

Table 1 Adjuvant activity of intact bacterial peptidoglycans and peptidoglycan fragments

µg	Intact peptidoglycan from				<i>E. coli</i> peptidoglycan fragments			
	<i>E. coli</i>	<i>S. faecalis</i> *	<i>L. casei</i> *	<i>S. epidermidis</i> †	Disaccharide peptide monomer	Peptide dimer	Peptide monomer	Disaccharide
100					3/3 (14)			
50	4/4 (15)	6/7 (12)	6/7 (12)	4/4 (16)	4/4 (12)			
10	4/4 (14)				3/4 (13)	7/8 (16)	3/4 (13)	0/4
1	2/4 (13)				6/8 (12)	7/8 (14)		
0.1	0/4				1/4	1/4		
0.01						0/4		
0	0/10							

The cell wall compounds were added to a solution of azobenzene arsonate-N-acetyl-L-tyrosine and the mixture emulsified in an equal volume of incomplete Freund's adjuvant (Difco). All the components were sterilised either by heating or by filtration (0.22 µm pore size). Random bred white guinea pigs (500–700 g) were injected into the four footpads with a total volume of 0.2 ml emulsion containing 2×10^{-7} M of antigen and the indicated amounts of adjuvant. Animals were skin tested 12 d later with 10 µg azobenzene arsonate–guinea pig serum albumin¹² and the diameter of skin reactions was measured 24 h later. Only skin reactions over 8 mm were recorded as positive. Results are expressed as the ratio of sensitised to treated animals. The average diameter of the skin reactions of sensitised animals are indicated in parentheses. Primary structure of the tetrapeptide units²⁰:



colipidic nature². Hydrosoluble adjuvants containing an arabinogalactan and the wall peptidoglycan have been isolated^{3–5}. We have shown previously that the purified cell wall peptidoglycan of several Gram-negative bacteria exhibit the same adjuvant effect as mycobacteria for the induction of delayed hypersensitivity^{6,7} and that the disaccharide peptide monomer (β-1,4-N-acetylglucosaminyl-N-acetylmuramyl-L-Ala-D-Glu — (L) diaminopimelic acid (L)-D-Ala) was still active⁸. Similar findings have been made independently with the peptidoglycan of mycobacteria^{9,10}, the subunit structure of which is only slightly different: N-acetylmuramic acid is replaced by N-glycolylmuramic acid and both Glu and diaminopimelic acid residues have amide substituents. The present study was undertaken to determine to what chemical structure of the peptidoglycan adjuvant activity was related.

Adjuvant activity was tested with monoazobenzene arsonate-N-acetyl-L-tyrosine as antigen. This compound induces a pure state of delayed hypersensitivity when injected in complete Freund's adjuvant^{11–14} but is nonimmunogenic in incomplete adjuvant¹².

The peptidoglycans containing L-lysine were isolated by the method of Schleifer and Kandler¹⁵ from the following Gram-positive bacteria: *Streptococcus faecalis*, *Lactobacillus casei* and *Staphylococcus epidermidis*. The peptidoglycan of *Escherichia coli*, its disaccharide peptide monomer and bis disaccharide peptide dimer (where two peptide monomers are linked together through a D-alanyl-(D)-meso-diaminopimelic acid linkage) were prepared as previously described¹⁶.

Hydrolysis of the disaccharide peptide monomer by the *Bacillus licheniformis* amidase¹⁷, and hydrolysis of the bis disaccharide peptide dimer by the *Streptomyces* N-acetylmuramyl-L-alanine amidase¹⁸ yielded disaccharides and peptides which were separated by filtration on Sephadex G10 and paper electrophoresis¹⁹.

Peptidoglycans containing L-lysine instead of meso-diaminopimelic acid also possess adjuvant activity and the peptidic fraction of *E. coli* peptidoglycan has the same activity as the intact polymer (Table 1).

From this the peptide units L-Ala-D-Glu — R₃-D-Ala, where R₃ is either a meso-diaminopimelic acid or a N^ε substituted-L-lysine residue, seem to be the smallest bacterial structures exhibiting adjuvant activity. It is likely that this kind of peptide is not easily degraded by animal cells and this may play a part in its adjuvant activity.

We thank Professor J. M. Ghuyssen for advice and for providing *Chalaropsis* B endo-N-acetylmuramidase and *Strep-*

tomyces N-acetylmuramyl-L-alanine amidase, and Mrs Ehling and Miss Gouzerh for technical assistance. This work was supported by grants from the Centre National de la Recherche Scientifique and the Institut National de la Santé et de la Recherche Médicale.

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book reviews

Quality quantified

Social Stratification in Science. By Jonathan R. Cole and Stephen Cole. Pp. xiv+283. (University of Chicago: Chicago and London, 1973). £5.65.

SINCE 1967 the Coles have been addressing themselves to a set of interesting problems concerning the internal social organisation of science, using as principal tool the *Science Citation Index*. The number of citations received is taken to be a measure of the quality of scientific work. Those who disdainfully dismiss this way of measuring quality should read the discussion of its reliability and validity in chapter 2 of the book. Despite the admitted drawbacks—notably the fact that important discoveries may become accepted to the extent that the original papers stop being cited—it emerges from the scrutiny as a reasonable indicator.

The Coles' major concern is the degree to which the reward system of science operates by universalistic criteria. How far do inequalities in the rewards received reflect quality of work, rather than particular attributes like sex or race or being related to the right people? Is the homage which conventional rhetoric pays to universalistic ideals better matched in practice by science than by other social institutions? The answer is reassuring. Science—which here means mostly American physics—emerges in a favourable light. For receipt of honorific awards, for visibility and for appointments in prestigious university departments, quality of research is found to be a key determinant.

Women are not much discriminated against—once they are over the Ph.D. hurdle. The same limitation effectively removes from the field of study the question of discrimination against blacks, because they receive so few Ph.Ds (less than one per cent of those given in science). The restricted scope of the Coles' study is exposed by their revealing use of the expression "social origins" to refer to the rank of the scientist's doctoral department (page 117). It is only for those who have got inside the system that its inequalities can be seen to be equitably distributed.

Is there a serious departure from universalism in what R. K. Merton has called "the Matthew effect" ("For unto every one that hath shall be given"—Gospel according to St Matthew)? The Coles test the consequences of such an

assumption for the diffusion of knowledge and conclude that the effect is rarely important. Controlling for assessed quality at time 2, the assessed quality of papers at time 1 is only slightly influenced by the reputation already achieved by their authors.

The Coles also test what they call "the Ortega hypothesis", after an assertion by Ortega y Gasset that the many scientists who are of no more than mediocre quality nevertheless contribute in a major way to scientific progress. The work that physicists use in their best papers, as indicated by the references they cite, is itself found to be produced largely by elite physicists. This does not support the hypothesis and leads to the suggestion, which may antagonise some readers, that the number of active physicists could be cut substantially without crippling progress.

Science as studied by the Coles is defined in a narrow way. Only the publication of research papers counts; even review articles are excluded. Teaching students or administering research establishments or advising governments is "unproductive"; so is improving the productivity of industry. The "quality" of a company or government agency is determined by its commitment to basic research (page 43). Social responsibility in science is outside this impregnable circle of definitions—so far outside that the idea that "rather than society influencing science, science influences society" is described as one that "some of the more polemical historians of science have gone so far as to argue" (page 14).

The most immediate danger arising from work like that described in this book is that statistical measures adequate for fair-sized samples may be misapplied to individual cases. Within a few pages of a warning about this (page 31), the authors themselves are teetering on the brink. In general, however, they remain clearly aware of the limitations of their approach and they use their tools with care as well as ingenuity. They have done a useful exercise in the art of the possible. Like it or not, citation counts will doubtless continue to be used as well as misused, and the Coles' work itself is likely to be plentifully cited. The smokescreen of sociological jargon in their book is refreshingly thin and the style is lucid, considering the high density of correlation and regression coefficients.

F. R. JEVONS

Ignorance of interferon

Interferon: Theory and Applications. By V. D. Solov'ev and T. A. Bektemirov. Translated from Russian by Basil Haigh. Pp. xvii+304. (Plenum: London and New York, 1974.) \$30.

THERE have been several international meetings on interferon during the past few years, but unfortunately Russian workers have not been able to attend any of them—often having to withdraw at the last moment. Since the Soviet Union has also had few visitors from the West, this has led to the isolation of the Russian workers and the effects of this isolation are only too apparent in this present book.

The book claims, in its forward, "to give a systematic account of the existing information on interferon obtained both from the extensive literature, and from the authors' own observations made over a period of several years..." The book falls sadly short in its first aim—that is to give a systematic account of the existing information on interferon. First of all, it is out of date. This is obvious as soon as one looks at the extensive list of references given at the end of the volume. The first hundred and ninety refer to Russian papers published up to 1967, the next four hundred to papers by non-Russian authors (only three being later than 1967), while the final hundred are made up of more recent Russian publications and Western papers, but only twenty odd are dated 1970 or later. This is presumably because the Russian text was published in 1970, but it is a pity that the text was not updated before translation. The book shows its age in other ways too—the account of interferon purification is nearly ten years old and data presented on the molecular weight and amino acid composition of interferon is quite incorrect. The translational-inhibiting protein theory of Marcus and Salb is restated although it was abandoned years ago. Isaacs's theory that interferon induction is due to 'foreign' nucleic acid is described, although Isaacs himself later withdrew it. Nagano's inhibitory factor is described as an interferon, despite Fantès's (1966) careful analysis of the results, showing that the inhibitory factor is almost certainly a polysaccharide. There is no account of the interferon standardisation meeting in London in 1969, nor of the development of

reference research standards for interferon.

The other aim of the book is to describe Russian work hitherto unpublished in English. This falls into two main areas—measurement of interferon production by different viruses in a variety of cells and the use of interferon in man. The interferon production experiments are presented at great length and quite uncritically, and their total effect is merely to demonstrate that many factors are responsible for the control of interferon formation. The work on the role of interferon in infection and its use in clinical trials in the Soviet Union is more interesting, and is the only justification for translating the book into English. I am much less competent to assess this data, which seems to me to be interpreted rather optimistically.

In summary, this is not a book to purchase or to recommend for library purchase, unless one has an interest in learning of Russian work on the role of interferon in natural infections.

D. C. BURKE

Technology of insect flight

Insects in Flight. By Werner Nachtigall. Translated by Harold Oldroyd, Roger H. Abbott, and Marguerite Biederman-Thorson. Pp. 150; 32 plates. (Allen and Unwin: London, May 1974.) £5.50.

MUCH that is new has been learned about insect flight in the past fifteen years, and Werner Nachtigall has been one of the most productive workers in this field. He has published a long series of papers in German which provide a detailed analysis of wing movements in the blowfly in free flight, by the use of highly sophisticated experimental methods. A few years ago he conceived the idea of writing a book about the flight of insects which would illustrate the thrills and frustrations of research, and form a tribute to the beauty and ingenuity of the technology of insect flight. The book has now been translated into English and appears in well printed and beautifully illustrated form.

The author writes for students and teachers with interests in technology and engineering, for those engaged in piloting gliders or powered aircraft, and indeed for the general reader. He is wholly successful in putting across the biophysics of the flying insect—though he expects his readers to concentrate! He describes the aerodynamics of flight in terms of instantaneous linear forces and avoids the more demanding aerodynamical approach: bound vortices and the generation of the aerodynamic cross force are passed over and Reynolds numbers appear only in a single caption. The story is varied and enlivened

by literary excerpts; by excursions into locust plagues; the rôle of flight in the life of the honey bee and other insects; insect migration; fuel, temperature and sensors in flight; the technological rivalry between sonar hunting bats and acoustically equipped moths; the contrasts and comparisons of insect and human flight—and much more besides.

The author has a gift for vivid depiction: “to a fly, a cine film must look like a lantern lecture, with long dark pauses in between the slides”. The book is admirably translated but in at least one place the popular error in translating ‘Schmetterling’ leads to the description of the hawk-moth *Celerio* as a ‘nocturnal butterfly’.

V. B. WIGGLESWORTH

Exploring evolution

Evolution in the Microbial World. (24th Symposium of the Society for General Microbiology, held at Imperial College London, April 1974.) Edited by M. J. Carlile and J. J. Skehel. Pp. x+430. (Cambridge University: London, April 1974.) £7.50; \$22.50.

THIS volume, in the words of the editors, is believed to represent the first account devoted to the topic of microbial evolution. Such an embracing title would be expected to cover a very wide range of topics indeed and, although there is a great deal of information in this book which bears on the evolution of microbes, it would be useful to indicate what the book does not contain. It does not include, and does not claim to cover, aspects of comparative microbial anatomy nor a discussion of phylogeny based on such evidence. In fact, no indication is given of the wide range of structural diversity found in microbes. Similarly, there is little reference to the possible route of evolution of major groups of microorganisms.

Instead, the emphasis of the text is placed, not upon the apparent path of evolution, but on those special properties of microorganisms which make them such favourable experimental material for exploring the mechanisms of evolution. The sequence of chapters is essentially hierarchical, starting with problems of taxonomy and phylogeny and leading to essays dealing with mutation, recombination and selection. Successive chapters deal with the evolution of molecules, with organisms, and with energy-yielding processes as well as with the evolution of a biological association (symbiosis).

The first chapter (by P. H. A. Sneath) is a lucid summary of current strategies for constructing the phylogenetic relationships of microbes and includes a full discussion of taxonomic principles. The following four chapters deal

with aspects of the evolutionary mechanism. Drake, for example, discusses the role of mutation in evolution and the measurement of mutation rates. Richmond and Wiedeman discuss plasmids in relation to bacterial evolution. Although the significance of plasmid-borne resistance factors is stressed, the basic theme of the essay is recombination mechanisms involving plasmids and their possible similarities to recombination in phages. The wider role of recombination in regulating breeding systems and the formation of new species is considered by Esser. Kubitschek summarises some of the material concerning selection pressures on bacteria grown in the environment of a chemostat.

One of the most powerful analytical techniques yet applied to the study of phylogenetic relationships is the technique of doublet analysis, by estimating the frequency of nearest-neighbour base sequences in DNA. Subak-Sharpe *et al.* give a full account of their studies on the reliability of the technique as applied to the DNA of bacteria and viruses. Experimental approaches to the problems of the evolution of enzymes and the way in which specificity of action is related to enzyme activity are discussed by Hartley. A similar theme is reiterated by Clarke, who summarises her work on the nature of adaptation of bacteria exposed to novel substrates in terms of the evolution of the requisite enzymes needed to utilise the substrate.

Three chapters are devoted to the evolution of energy-yielding processes: photosynthesis (Stanier), sulphur metabolism (Peck) and nitrogen fixation (Postgate). These essays do not merely summarise the salient features of the relevant biochemistry but also discuss the possible origins and evolution of such mechanisms in prokaryotes and eukaryotes. The remaining chapters are devoted to patterns of organisation and association among microbes. As examples of the evolution of organisms two divergent levels of organisation are considered: viruses (Joklik, Skehel) and the haemoflagellate *Trypanosoma* (Baker). The nature of symbiosis and mutualism are discussed by Lewis. Finally, Ponnamburuma and Gabel discuss the possible condition of the pre-biotic milieu in relation to the formation of complex molecules and the emergence of life.

As a summary of current thinking on problems of evolution among microbes this volume contains much to interest the reader. More importantly the essays included succeed in demonstrating the feasibility of using microorganisms to unravel some basic aspects of the evolutionary mechanism.

I. D. J. BURDETT

Mouths of waders

Feeding and the Feeding Apparatus in Waders: A Study of Anatomy and Adaptations in the Charadrii. By P. J. K. Burton. Pp. 150. (British Museum (Natural History): London, 1974.) £3.50.

DURING recent decades workers may have been somewhat deterred from studying the Charadriiformes by the documentation provided in "Witherby" and by the detailed knowledge possessed by amateur ornithologists but there is now evidence of a widespread resurgence of interest. This present book, a quite admirable discussion of functional morphology, considers charadrioid bill structure and oral musculature from a variety of viewpoints.

The information on overt feeding behaviour which is contained in the opening sections includes many original observations alongside a useful panoramic survey culled from the world literature. I was particularly interested in the statements about *Pluvialis squatarola* as they contrast with the data obtained by Dr G. A. Parker and myself. The remainder of the book deals in detail with the degrees of rhynchokinesis, and the variations in bill, tongue and hyoid structure, which are associated with different methods of feeding. In particular Dr Burton emphasises that highly rhynchokinetic upper jaws have probably evolved independently in at least seven lines of Charadriodea. The interdependence of modifications for a particular method of feeding is certainly widely known but this account provides many further examples.

Written with great clarity, the book will be a valuable addition to specialist libraries and encourage a continuing synthesis of morphology and ethology. One can only express passing regret that, although the work was initially supported by the Scientific Research in Schools Committee of the Royal Society, the author gave up his teaching post to complete it. Surely this was not the intention behind the committee? Britain needs able and enthusiastic teachers.

RONALD PEARSON

Seabed skeletons

Recent Sedimentary Carbonates. Part 1: Marine Carbonates. By J. D. Milliman. Pp. xv + 375 + 39 plates. (Springer-Verlag: Berlin and New York, 1974.) DM66; \$27.10.

THIS book is an excellent attempt to synthesise present knowledge about calcium carbonate in the marine environment, its composition, sedimentation and diagenesis. It is apparent that Milliman has used his experience

gained in researches over a broad spectrum of the field of Recent sediments and also a wealth of data from the latest literature to put together this comprehensive textbook, which will appeal to final year undergraduates, research students and established carbonate sedimentologists.

The book is divided into four parts: introduction, carbonate components, marine carbonate sedimentation and carbonate diagenesis. Part 1 includes a valuable introduction to the techniques employed in the analysis of such parameters as texture, petrography, mineralogy and elemental and stable isotope composition. This practical aspect is supplemented by the two appendices, which, although very brief, do establish a starting point for the identification, in thin section and under reflected light, of the major carbonate components of Recent marine sediments. The major portion (part 2) of the book synthesises an extensive literature on the ecology, calcification, petrography and composition of various skeletal and non-skeletal carbonate components. This section includes many quantitative data on topics including growth rates, mineralogies, minor trace element and stable isotope distributions which will serve as a useful reference for marine sedimentologists. The third part of the book deals with the distribution of marine carbonates. Milliman does not concentrate on a few shallow tropical sea environments, which is sensible, for this particular setting is treated in considerable depth in other recent books, but instead he gives equal attention to the shallow seas, shelf waters and the deep sea. The final part traces the diagenetic alteration of carbonates within the marine environment through degradation, cementation and dolomitisation.

The style is clear and the presentation straightforward. One of the most useful and original features of the book is the plentiful tabulation of quantitative and descriptive data from a wide variety of sources. In this way such information as chemical composition of skeletons, distribution of major planktonic foraminifera species, and depositional environments and chemical properties of modern marine dolomites are succinctly expressed for easy comparison and assimilation. The photographs are arranged in plates which on the whole are clear and appropriate, though for ease of reading I would have preferred photographs interspersed with the text. Several photographs are too small to serve much value and the general standard of plates is not the excellent quality one has come to expect from Springer-Verlag geological publications.

It is inevitable that this book will be compared with the recent comprehensive textbook on carbonate sediments and their diagenesis by Bathurst.

Though the themes of the two books are similar the contents overlap to a relatively small degree (as an indication only 375 references are common to the two books which have a total of over 2,000 references). This partly results from the different experiences and approaches of the two authors and partly from the inclusion in Milliman's book of many references to works published during the past three years.

T. P. SCOFFIN

Explaining solid state

Electronic Properties of Crystalline Solids: An Introduction to Fundamentals. By Richard H. Bube. Pp. xiii + 524. (Academic: New York and London, January 1974.) \$35; £16.80.

THE solid state physics contained in this book is, at first sight, fairly traditional, both as regards subject matter and treatment. On closer examination, however, it becomes apparent that Dr Bube has made a useful and significant addition to the range of text books now available to third year undergraduate and first year postgraduate students specialising in solid state physics. The author clearly intended to explain the main elements of the subject within a sound mathematical framework. He wisely resisted the temptation to discuss many aspects superficially and instead concentrated on taking a few examples of solid state phenomenon and treating them in some depth. Reviewers who judge a book by what has been left out may, therefore, find the choice of material not to their liking; I, for example, would like to have seen a little more on metallic alloys. But these are matters for personal judgment and should not be allowed to detract from a well written and beautifully produced textbook.

There are twelve chapters in the book which cover the three principal themes of solid state physics. The first three chapters focus attention on wave theory and the application of quantum mechanics to simple systems. The examples here were carefully chosen with an eye to later chapters. The next three chapters develop the band theory of solids taking as a starting point the free electron (Hartree) model. The reader will, if he works through these chapters and the excellent range of problems associated with each of them, gain a good understanding of basic band theory. I was particularly pleased to see a discussion of 'bands' versus 'bonds' and that the concept of electronegativity is at last appearing in solid state physics textbooks. The role of localised energy levels leading to the characteristic behaviour of semiconductors is described, a shade too briefly perhaps, in chapter 9. The remainder of the

book deals with the motion and excitation of carriers by external fields and includes a particularly helpful discussion of photoelectron effects. The book is well indexed, remarkably free from typographical errors and will, I feel sure, be a useful textbook for a variety of students (and their seniors) for the next few years.

J. E. ENDERBY

Lichens

The Lichens. Edited by Vernon Ahmadjian and Mason E. Hale. Pp. xiv+697. (Academic: New York and London, January 1974.) \$35; £16.80.

ABOUT one fifth to one quarter of all described species of fungi are lichenised, but very few books have been written about them. Indeed, this is the first large and comprehensive work of advanced scholarship in the English language about lichens since the classic monograph of A. L. Smith in 1921. Furthermore, since so few botanists specialise in these plants, there is unlikely to be another book of this type for some time to come. It is, therefore, something of a milestone in the development of the subject.

There are 23 authors contributing 19 chapters and 3 appendices, covering a wide range of topics including morphology, taxonomy, reproduction, physiology, ecology, secondary metabolic products and symbiont interactions. Inevitably, there are some gaps. In the preface, the editors say that chemotaxonomy is one of the main present day areas of research in lichens, yet the book has no chapter devoted to this richly controversial topic: indeed, it gets but a passing mention in one appendix, and is not cited at all in the index.

Unevenness in quality is to be expected in all multi-author works, and this is no exception. Although the editors have been very thorough and painstaking—so that errors and unclear passages are rare—it is a pity that they did not persuade authors to be more interpretive in their approach. Too many contributors are content to give meticulous accounts of published observations without following up with an incisive section which says, in effect, "... now what all this means is ... so that the problems which now require solution are ...".

Hence, the memorable parts of this book are the handful of chapters in which the authors develop a critical, personal synthesis of their views. Poelt's critique of the systematic value of morphological characters is particularly good. The account by Tuominen and Jaakola of the accumulation of mineral elements and radionuclides is

not only a valuable review of widely scattered literature, but it also sets a refreshingly high standard of rigour in discussing physiological topics. Brodo's excellent chapter on substrate ecology is profoundly thoughtful: at long last, here is a lichen ecologist who can write, quite simply, "I think the terms 'nitrophilous' and 'calciphilous' imply a knowledge of the requirements of lichens that we do not yet have".

For some students, the book will be hard going for it lacks an introductory chapter to set the scene. More acutely, it needs a concluding chapter to draw together the diverse strands of the individual contributions. Here would have been a chance for the editors to paint the broad canvas of the nature of the interactions between the symbionts; or to integrate the different themes of the chapters on substrate ecology, resistance to extreme environments, and mineral accumulation; or point out the gaps which, in the preface, they say exist.

The price puts this book beyond the reach of almost all students and academics, but I think it is essential for all botanical libraries. Although it does not achieve the same classic status as A. L. Smith's 1921 monograph, it nevertheless contains some important, substantial and valuable contributions about lichens which are not available elsewhere. Academic Press may be able to blame inflation for the price, but they cannot blame anyone but themselves for a subject index which is so skimpy, has so very many omissions and is so poorly organised as to be nearly worthless.

D. C. SMITH

Blue-eyed Negroes

Light-Eyed Negroes and the Klein-Waardenburg Syndrome. By Jenni Soussi Tsafir. Pp. viii+153. 20 plates. (Macmillan: London and Basingstoke, March 1974.) £7.50.

THIS little book describes and discusses 18 non-Caucasian families ascertained by eye colour, where one or more members had Waardenburg's syndrome, and 10 families where one or more members had unilateral or bilateral blue or light green eyes, or heterochromia iridis.

Much of the book is devoted to discussion of Waardenburg's syndrome and, though the clinical descriptions of manifestations of the disorder are excellent, there is much repetition and nothing new is added to knowledge or understanding of an autosomal dominant trait which is relatively common and has been reported in many races. The families where there was only hypochromia of iris, which resulted in blue or greenish eye colours, or

where there was heterochromia inherited as a dominant trait are of much greater interest, and this is the best account so far given of these phenomena in Africans.

I feel that all the information in the book could have been presented in a short paper, or possibly in two papers, one on Waardenburg's syndrome in Africans and one on the other group of subjects. As it stands the book reads as if it was a condensation of an excellent thesis, although such an origin is not mentioned. Presumably the very high cost of this book is in part due to the nine pages of excellent colour photographs.

ALAN C. STEVENSON

African medicine

La Pharmacopée Sénégalaise Traditionnelle: Plantes Médicinales et Toxiques. By J. Kerharo, with J. G. Adam. Pp. 1011. (Editions Vigot Frères: Paris, 1974.) 370 francs.

PROFESSOR Kerharo is well known for his previous studies on the indigenous medicine and medicinal plants of the Ivory Coast and Upper Volta. During the past 15 years he has carried out similar, very detailed investigations in Senegal and his findings are now made available in this monumental work. The first part (about 75 pages) fills in the historical, (phyto) geographical, and ethnic background. It also discusses the religious and magical concepts underlying the various indigenous systems of medicine as well as the more positive aspects like diagnosis, pharmaceutical operations, and how medicines are dispensed and administered.

The second and main part of the book (about 700 pages) comprises detailed, individual monographs on about 555 plants. These monographs are divided into sections dealing with synonyms, vernacular names, distinguishing botanical features, habitat, folk-medicinal uses, chemistry and pharmacology. The chemical and pharmacological sections are extended and up to date accounts which summarise data from more than 2,200 references (closing date, May 1973). They are a particularly important feature of the book and extend its usefulness and value far beyond the confines of Senegal, since much of the flora of that country occurs in other parts of West Africa and elsewhere.

The third part (about 200 pages) comprises the bibliography and the various indices. Professor Kerharo's book provides a welcome complement to Watt and Breyer-Brandwijk's *Medicinal and Poisonous Plants of Southern and Eastern Africa*.

N. G. BISSET